Durham E-Theses

An experimental taxonomic study of Chrysanthemum leucanthemum L.

Pearson, P. L.

How to cite:

Pearson, P. L. (1967) *An experimental taxonomic study of Chrysanthemum leucanthemum L.*, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/8735/

Use policy

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:

- a full bibliographic reference is made to the original source
- a link is made to the metadata record in Durham E-Theses
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the full Durham E-Theses policy for further details.

Academic Support Office, The Palatine Centre, Durham University, Stockton Road, Durham, DH1 3LE
e-mail: e-theses.admin@durham.ac.uk Tel: +44 0191 334 6107
http://etheses.dur.ac.uk

AN EXPERIMENTAL TAXONOMIC STUDY OF CHRYSANTHEMUM LEUCANTHEMUM L.

A thesis submitted for the

degree of

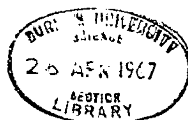
Doctor of Philosophy

by

P. L. Pearson

St. Cuthbert's Society,
The University of Durham.

Lent Term,
1967.



An alpine variety of Chrysanthemum leucanthemum L.
from the Central Pyrenees.

ACKNOWLEDGEMENTS

I wish to acknowledge Professor D. H. Valentine for being an encouraging and helpful Supervisor; The Science Research Council for providing a research grant and maintenance allowance for my dependents; colleagues for helpful criticisms and suggestions and supplies of plants; Dr. A. C. Stevenson, Director of the Medical Research Council Population Genetics Unit for being patient while this thesis was being completed; and my wife for typing the script.

TABLE OF CONTENTS

Section I.	Cytological variation in *Chrysanthemum leucanthemum* L. sensu lato.	Page 1.
Section II.	Geographical and Ecological Variation in *Chrysanthemum leucanthemum* L. sensu lato.	Page 55.
Section III.	An analysis of Breeding Behaviour in *Chrysanthemum leucanthemum* L. sensu lato.	Page 80.
Section IV.	Variation of Phenolic Pigments in *Chrysanthemum leucanthemum* L. sensu lato.	Page 87.
Section V.	Morphological Variation in *Chrysanthemum leucanthemum* L. sensu lato.	Page 92.
Section VI.	Taxonomical and Nomenclatural considerations in *Chrysanthemum leucanthemum* L. sensu lato.	Page 121.
Section VII.	General Discussion	Page 132.
Appendix I.	A list of chromosome counts and the origin of the plants used in the present work.	Page 134.
Appendix II.	A list of chromosome counts and origins of the material used in previously published works.	Page 140.
Appendix III.	Details of the various cytological techniques used in the present work.	Page 144.
Appendix IV.	Comments on the technique of chromosomal photomicrography.	Page 147.
Appendix V.	Karyotype analysis in *Chrysanthemum leucanthemum* L. sensu lato.	Page 150.
Appendix VI.	Printout of program and the results of the autotetraploid meiotic pairing routine.	Page 169.
Appendix VII.	Species list taken at Localities 1 and 2 in Vallée de Rio Esera, Central Pyrenees.	Page 174.
Appendix VIII.	A species list taken at the Limestone Cliff localities of the Great Orme and Humphrey Head.	Page 176.

TABLE OF CONTENTS cont.

Appendix IX.	A species list taken at Cwym Idwal	Page 177.
Appendix X.	The Thin Layer Chromatography Method used.	Page 178.
Appendix XI.	Randomised growth experiment.	Page 180.
Appendix XII.	Methods and Assumptions used in the Multivariate analysis.	Page 181.
Appendix XIII.	Details on the uses and specifications of the Numerical Taxonomic Programs.	Page 181A.
Appendix XIV.	Characters used in the Multivariate Analysis.	Page 196.
Appendix XV.	Statistical significance and distribution properties of the correlation coefficient when used in numerical taxonomy.	Page 202.
Appendix XVI.	Some morphological comparisons made between four populations.	Page 205.
Appendix XVII.	Program and data printouts of the programs used in the multivariate analysis.	Page 206.
Bibliography		Page 216.

SECTION I

CYTOLOGICAL VARIATION IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

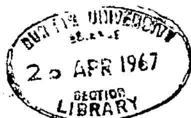
INTRODUCTION

Within the last few years considerable interest has been generated in the use of cytological characters for assisting in taxonomic treatments of the Composite species Chrysanthemum leucanthemum L. sensu lato. This species is extremely variable and numerous infra-specific taxa have long been recognised. Recent works (see following text) using North American and Continental material have suggested that there might be sufficient grounds for splitting up the species aggregate into specific taxa corresponding to chromosome races.

Although on the basis of their distribution of chromosome races on the Continent, Böcher and Larsen (1957) have surmised that only the diploid chromosome race grows in the British Isles, the situation is in fact, unknown. In this context Valentine (1961) has stated, "It is too early to be certain whether all British forms of this group belong to the diploid species, and further investigation is needed."

In the following account I have attempted to analyse certain aspects of variation within the species aggregate using a variety of techniques. The enormity of the task has prevented representative samples from the whole geographical and morphological range of the species aggregate from being considered. As a result of this my attention has been primarily confined to variation within British material and diploid and tetraploid chromosome races of Continental material.

NOTE. The term 'population' has been used throughout the text to refer to groups or samples of plants which have a close spatial relationship with each other and are distinguished from other populations by the presence of obvious discontinuities in that relationship. No genetical implications have been attached to the term 'population'.


2 APR 1967
LIBRARY

CYTOLOGICAL VARIATION IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

Review of the literature

Tahara (1915, 1921) first reported the tetraploid number of 2n = 36. Subsequently, Orth (1926), Shimotomai (1938) and Dowrick (1952) published counts of 36 for the species; Polya (1950) published the first diploid count of 2n = 18 from Hungary and in 1956 Duckert and Favarger found 2n = 18 in an alpine population from the Jura. In the following year, Böcher and Larsen (1957) gave a cytotype distribution of the species on the basis of some thirty locality counts including a hexaploid count of 2n = 54. Baksay (1957) demonstrated the existence of diploid, tetraploid and hexaploid plants in the Tatra of Hungary and this was followed by a paper by Favarger (1959) on the distribution of chromosome races within Switzerland. Skalinska and her co-workers (1961 & 1963) found a comparable distribution of chromosome races in Poland to the Hungarian and Swiss ones of Baksay and Favarger, respectively. Practically all the work cited above was confined to mitotic counts without examination of cytological variation other than chromosome number.

There has been very little work published on meiotic chromosome variation in Chrysanthemum leucanthemum L. or in the rest of the genus for that matter.

Cooper and Mahony (1935) published a count of n = 18 on material taken from the Campus, University of Wisconsin, in which they found 18 bivalents. Dowrick (1952), while working on chromosome variation

within the genus, noted that in general the tetraploids showed some degree of multivalent formation and concluded that the most probable origin for polyploids was by autopolyploidy. He did not make the essential distinction between homologous chromosome associations and associations resulting from interchanges. In an examination of tetraploid meiotic material of Chrysanthemum leucanthemum L. he made the important discovery that some anaphase 1 cells contained 36 chromosomes at each pole. This resulted in diad formation and pollen with 36 chromosomes. Whilst examining the meiotic chromosomes of tetraploid Chrysanthemum atratum L. Dowrick discovered the same phenomenon of non-reduction of pollen and attributed it to the precocious splitting of the centromeres at anaphase 1. He suggests that it is the capacity of the centromeres for further division which determines whether there is a second division or not. Although the examples described refer to tetraploids, it is quite possible that the same phenomenon could arise in diploids and hence be a possible source of spontaneous autopolyploidy.

Favarger and Duckert (1956) described the meiosis of a high alpine diploid form of Chrysanthemum leucanthemum L. as being anomalous. Several indeterminate line drawings and photographs are given in the text which add little to the evidence for an anomalous meiosis. In his 1959 paper on Chrysanthemum leucanthemum L. Favarger said that the abnormal diploid meiosis described in 1956 was probably confined to the locality from which he sampled and that all subsequent investigations on other material had revealed normal bivalent pairing. On the

tetraploid chromosome pairing, he comments that although he has no counts of his own, previously published work indicates that pairing is regular and hence indicative of an allopolyploid origin. All published counts to date are given in Appendix II.

Mitotic chromosome variation

Root tip analyses were made on potted plants using the methods described in Appendix III. All the plants of natural origin examined were found to have numbers on the 2n, 4n or 6n levels of ploidy, thus confirming the counts of previous authors. Other chromosome numbers, as found by Favarger (1959), Villard and Favarger (1966), were not detected. A list of material counted is given in Appendix I. Careful observations on intraplant chromosome variation revealed that the chromosomes were all more or less the same length and that the centromeres were either median or submedian in position. Satellites and secondary constrictions were occasionally observed, but were most irregular as regards visibility. The main factors which determined this irregularity appeared to be excessive chromatin contraction due to excessive pretreatment and injudicious squashing during slide preparation. In spite of these handicaps, it was decided to carry out an analysis to try and determine whether the observable, slight morphological differences could be interpreted in terms of interplant, inter population, and interploid variation.

It was thought at first that making camera-lucida drawings of

chromosomes and taking measurements from the drawings would be adequate, but it was found that the inaccuracies introduced by an inability to reproduce a chromosome outline precisely by means of a pencil line were too great relative to chromosome size, and the more sensitive procedure of making photomicrographs of standard enlargement and either pairing and/or measuring the chromosomes from a bromide print was adopted. This procedure requires that all the chromosomes be in the same focal plane to minimise the errors of converting an essentially three dimensional light image into an observed two dimensional distribution of silver grains. Naturally, the success of the process is also dependent upon adequate resolving power of the optical system and the photographic techniques employed.* As already pointed out there are no particularly good mitotic chromosome markers in Chrysanthemum leucanthemum L. and it would be useful to examine the variation pattern of each parameter individually to assess their taxonomic value.

For chromosome variation to be of taxonomic value one has to know whether identical chromosomes from different cells and plants consistently maintain the same position in the observed spectrum of variation for a cell. This necessitates being able to identify positively a particular chromosome in every analysable metaphase available. Such a chromosome was detected in diploid seedlings germinated from seed

---

* N.B. In Appendix IV can be found details of the photomicroscopy and the rationale for the various techniques used.

taken at Polzeath in Cornwall. See figs. 1 and 2, page 7. It is interesting to note that of thirteen seedlings counted, three were homozygous for the marker chromosome and ten heterozygous. This leads to the most likely conclusion that the seed was taken from one plant homozygous for the marker chromosome and that the plant concerned was outbreeding. The possibility that the parent plant was heterozygous and inbreeding cannot be excluded but is less likely from the observed frequencies. The chromosome concerned is one of the longer ones and has a subterminal centromere. The nature of this heterozygosity is unknown (meiotic material having not yet been examined), but it is likely to be a pericentric inversion.*

The value of such a chromosome is that not only can size variation be studied but also arm ratio and the effects of differential contraction upon different lengths of chromatin. On the basis of this, a coefficient of variation for the chromosome can be computed and this can be used to assess the confidence limits for homologous chromosome identification on the basis of long arm and short arm measurements.**

** The extrapolation of a coefficient of variation computed for one chromosome to others in the same genome does not appear to be a gross liberty. Patau (1964) has calculated individual coefficients for length variation of various human chromosomes and found them all to be about 5% irrespective of the chromosome concerned. The inference is that D.N.A.varies according to the same properties, no matter what size or shape of the chromosome concerned is. i.e. as regard contraction and stretchability.

* This material was kindly provided by R. A. Finch of the Botany Department, Oxford.

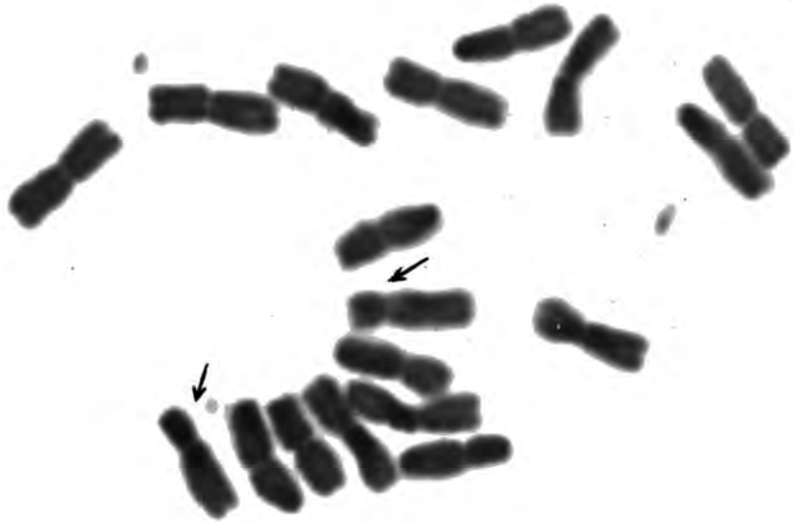


Fig. 1. Cell homozygous for marker chromosome indicated by arrows

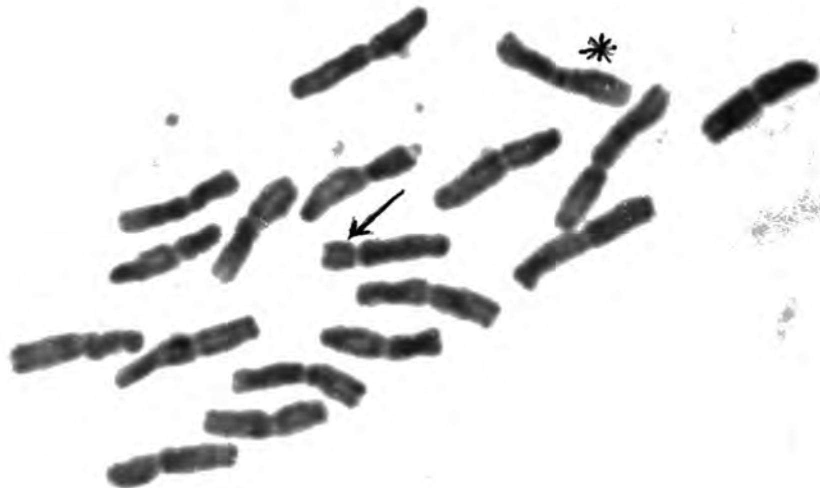


Fig. 2. Cell heterozygous for marker chromosome indicated by arrow
Secondary constriction indicated by asterisk.

Chromosome Size

Dowrick (1952) has suggested that in the Chrysanthemidae, chromosome length is negatively correlated with increase in polyploidy. Comparative measurements are difficult to make owing to differences in contraction, but maximum to minimum lengths for both diploid and tetraploid chromosomes are not significantly different.

	Maximum length	Minimum length
2x longest chromosome	8.0 microns	5.5 microns
4x longest chromosome	7.5 microns	5.5 microns
2x shortest chromosome	3.5 microns	3.5 microns
4x shortest chromosome	3.5 microns	3.5 microns

However, tetraploids appear to have a larger proportion of small chromosomes in their complement. This would imply that there is less than twice the amount of D.N.A. in tetraploid nuclei than there is in diploid nuclei. A crude estimate of D.N.A. content can be reached by measuring the length and breadth of interphase nuclei from squashed preparations. The assumption is made that if sufficient nuclei are measured then on average the thickness of nuclei will be more or less the same and that length and breadth provide sufficient parameters for D.N.A. quantity estimation. (fig. 3, page 9). Where the nucleus is oval, an over-estimate of the true area by a factor of $\pi/4$ is derived.

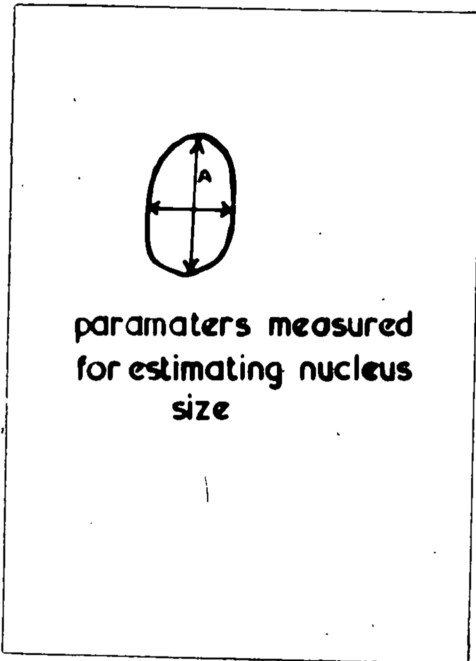


Fig. 3.

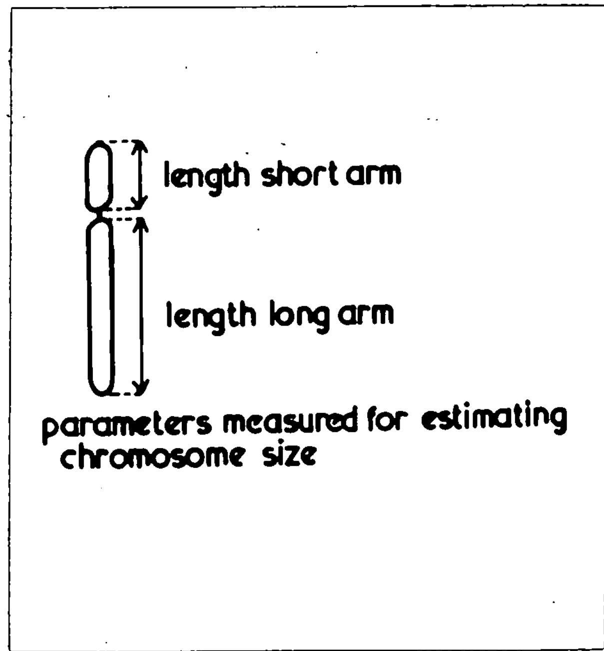


Fig. 4.

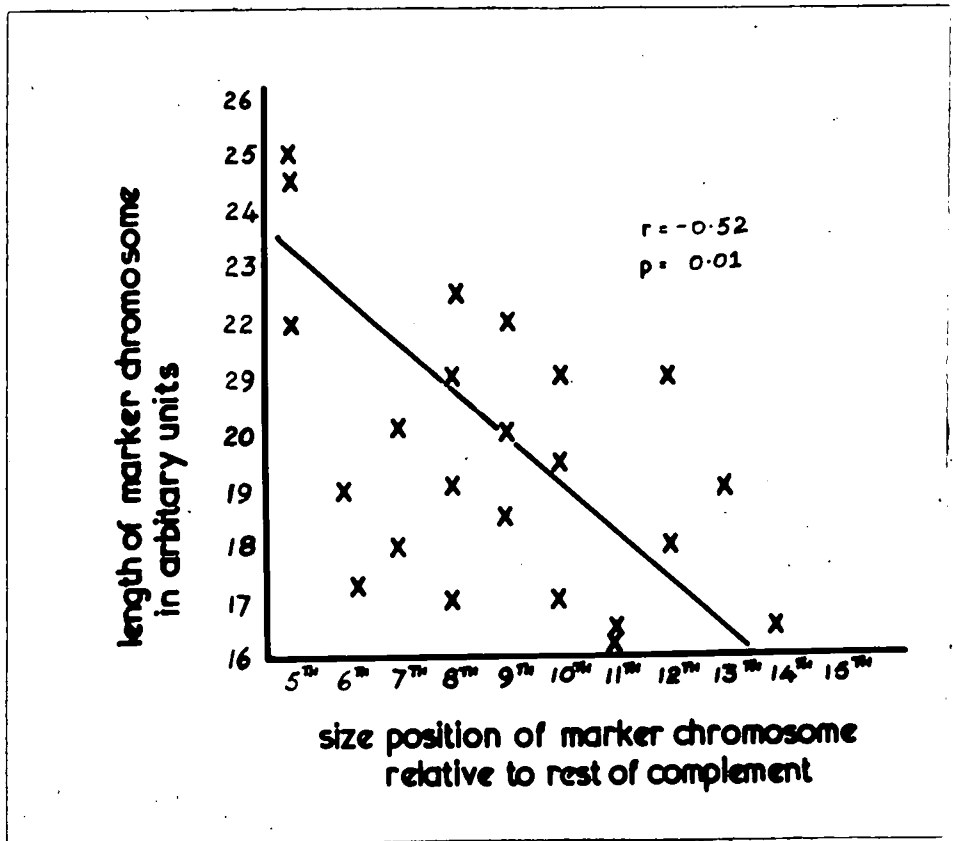


Fig. 5.

(This technique has also recently been used for estimating the quantity of D.N.A. in Barr Bodies and relating this to differences in X-chromosome size. Taft, P.D. et al. (1965). Klinger, H.P. et al. (1965), have increased the accuracy of the method by estimating nucleus thickness by Feulgen-microphotometry.)*

Fig. 6, page 11 shows that there appears to be a reduction in average size and hence D.N.A. content below the expected amount when comparing tetraploid nuclei with double that of the average size for diploid nuclei. However, the large standard deviations for the measurements mean that there is only just a significant difference between the observed and expected values for D.N.A. content. Estimations of D.N.A. content using Feulgen-microphotometry would reduce the standard deviations considerably and perhaps help to demonstrate the slight difference indicated above on a sounder basis.

In fig. 5, page 9 is plotted the length position of the marker chromosome compared with the other chromosomes in the complement against the absolute length of the marker. It can be seen that the marker chromosome varies in position from fifth largest to fourteenth in the complement. The length is regarded as being the long arm + the short arm length with an allowance made for the centromere position as shown in fig. 4. page. 9. The scatter indicates a negative correlation

---

* Dowrick at Wye College is at present researching into D.N.A. content in the Chrysanthemidae using the much more critical method of Feulgen microphotometry (personal communication).

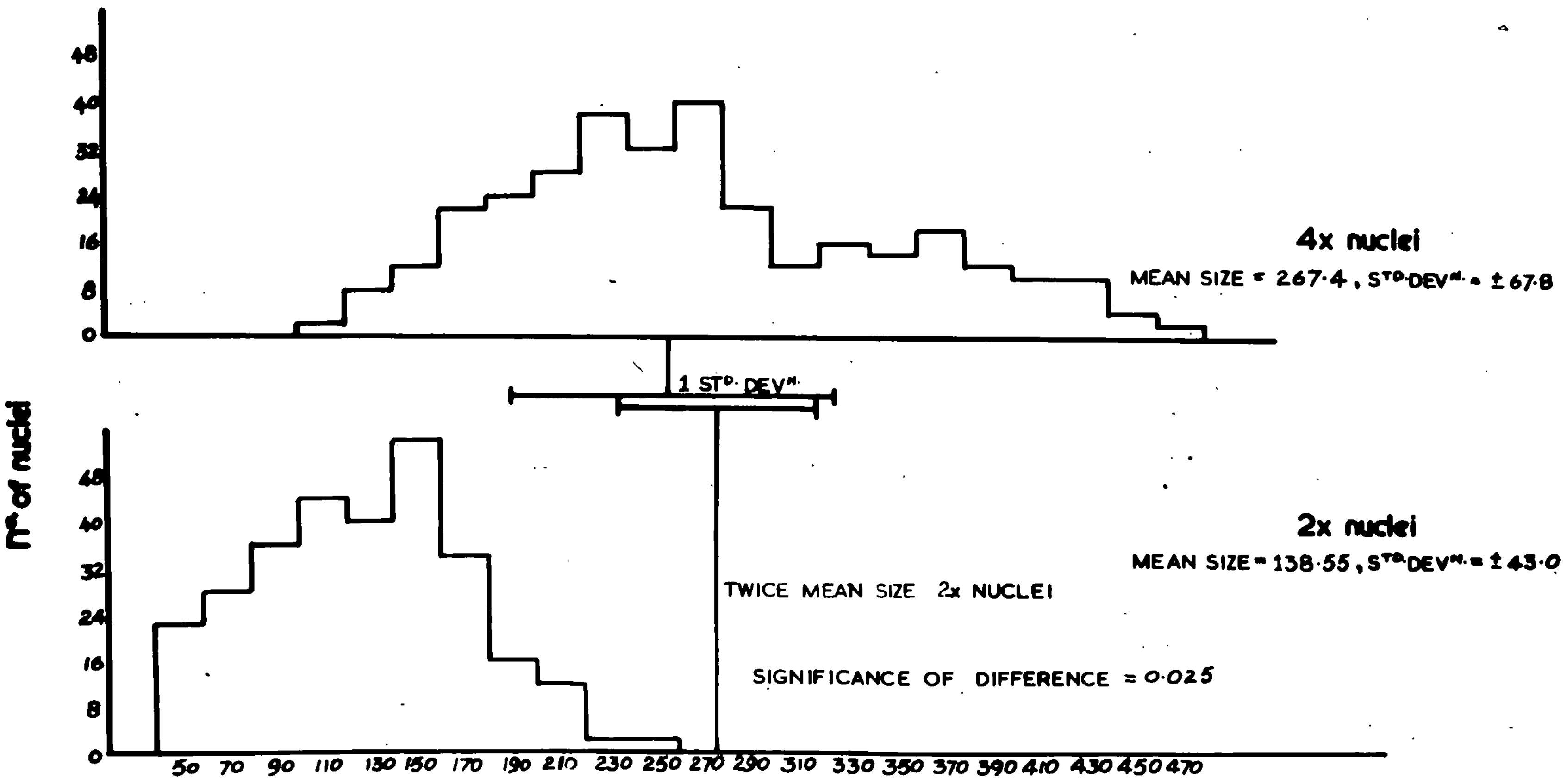


fig.6

between them. The correlation is − ·52, which is significant at the .01% level. The reason for this curious relationship is the apparent length polymorphism of the marker chromosome relative to other members of the complement, a point which will be discussed later.

Centromere position.

The position of the centromere is a cardinal character used in chromosome discrimination. As already noted, except for the marker chromosome, the chromosomes in Chrysanthemum leucanthemum L. tend to be meta- to submetacentric. In fig. 7, page 13 is plotted % length long arm against % length short arm for the marker chromosome. As expected there is a strong positive correlation, but by no means a perfect one, which could indicate that the short arm is not increasing in length in direct proportion to the long arm. In fig. 8, page 13 is plotted the ratio of % length long arm to % length short arm against % length marker chromosome. The correlation of x upon y for this scatter is −0.50 which is significant at the .01% level. This indicates that as the marker chromosome increases in length relative to the rest of the complement, then the ratio of long arm to short arm decreases, which would mean a polymorphism of the chromosome. As the measurements were taken from cells from several plants, this polymorphism could well exist between different plants.

The degree of variability of the marker chromosome was computed using a coefficient of variation, Patau (1964), where the coefficient

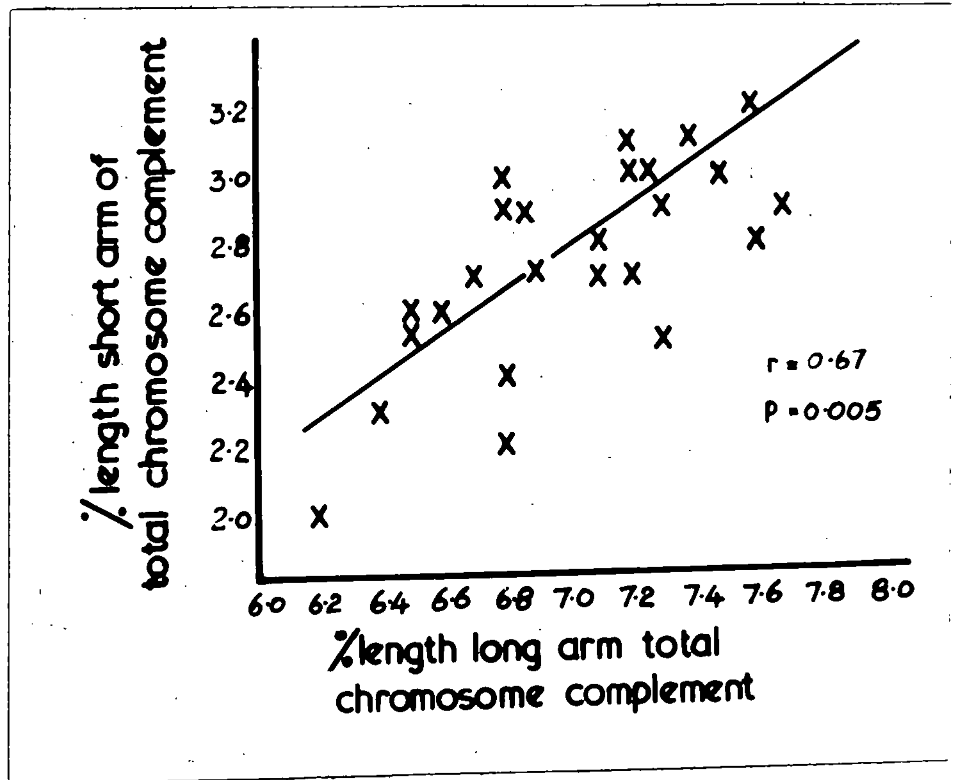


Fig. 7.

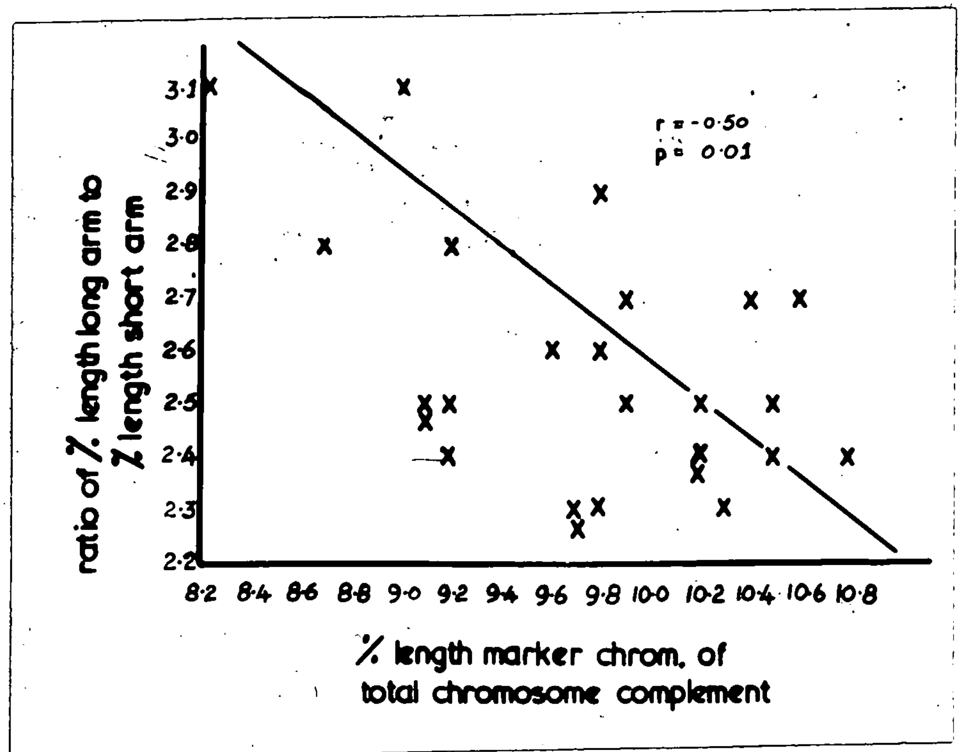


Fig. 8.

$$= 100 \quad 2n \sqrt{\sum (x_1 - x_2)^2} / \sum (x_1 + x_2)$$

and $x_1 = \frac{\text{length long arm}}{\text{length short arm}}$ x total autosome length

for one cell and x_2 the same parameter for another cell. The comparison was made between every cell and the value of the coefficient was 6.8%. This variation is of the same magnitude as that computed for chromosomes of other organisms. If the assumption is made that other chromosomes within the complement can vary to the same extent (a not unwarranted decision as pointed out on page 6) then the lack of positive variation between chromosomes probably means that chromosomes cannot be consistently identified on the basis of length and centromere position alone.

Satellites and Secondary Constrictions

Very few cells present the chromosomes in a form suitable for secondary constriction and satellite analysis, probably due to the contracting action of spindle inhibitors. A conservative estimate of the number of good cells necessary to establish the position and number of such chromosome markers is twenty or more in *Chrysanthemum leucanthemum* L. The maximum number of satellited chromosomes observed in a diploid plant is six, fig. 9, page 15 , and eight in a tetraploid plant. Careful examination showed that the marker chromosome had a small satellite on its short arm but this was only observed in five cells.

Fig. 9. British diploid with five satellited chromosomes indicated by arrows. A sixth is present but is not visible in this cell.

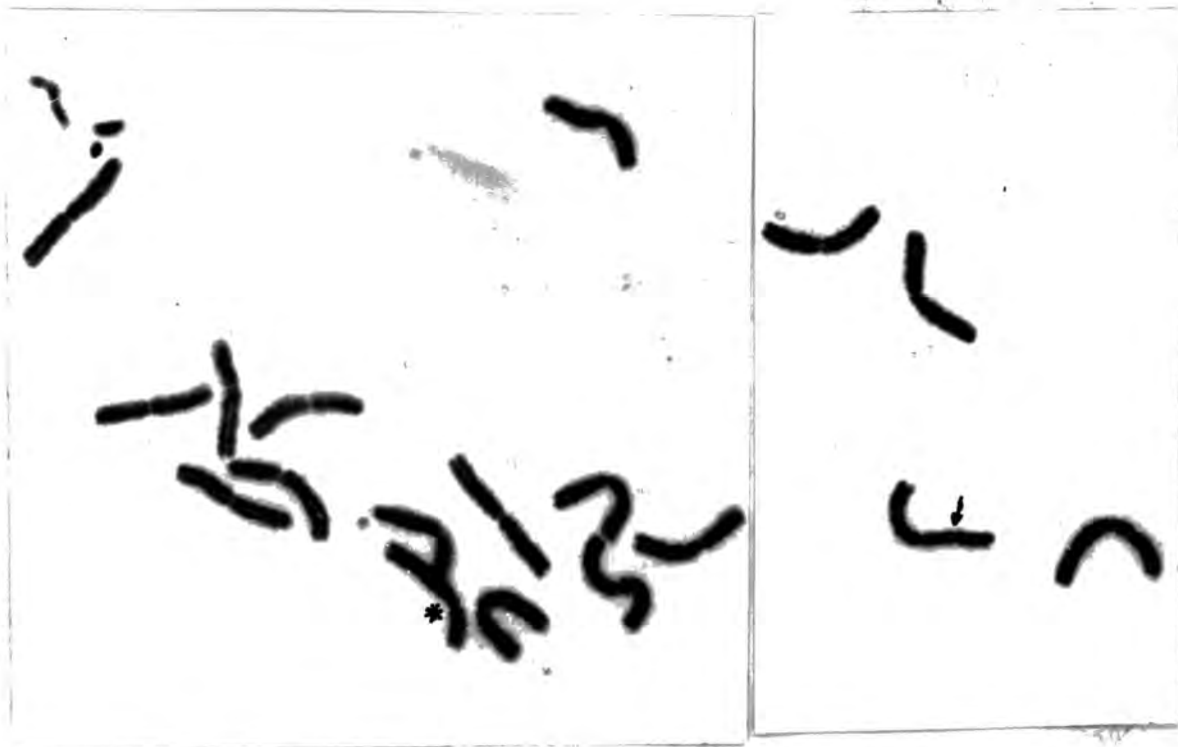


Fig. 10. British diploid with a chromosome with secondary constriction in long arm indicated by arrow. A chromosome identical to the Cornish marker indicated by asterisk.

The only secondary constriction which could be detected in several diploid populations was one which divided the long arm into two equal parts, Fig. 10, page 15. Three tetraploid plants from Derbyshire showed a similar secondary constriction in one chromosome. Fig. 2, page 7, shows a chromosome with a marked secondary constriction in juxtaposition to the centromere. This chromosome was present in some of the Cornish populations examined.

In Appendix V is a set of karyotypes constructed for diploid, triploid, and tetraploid cells. No assertions are made as to the "correctness" of a particular arrangement other than it appeared to be the best one for the cell concerned. Because of the uncertainty of chromosome identification on these criteria, a generalised idiogram has not been constructed for the chromosome complement of _Chrysanthemum leucanthemum L_. A tentative idiogram has been derived for British diploids. This is given in Appendix V.

The following general points emerge from the mitotic chromosome examination.

1. With the exception of the marker chromosome no other chromosome can be consistently recognised in either diploid or tetraploid _Chrysanthemum leucanthemum L_. although examination of many cells permits one to recognise at least three others in diploids.

2. Diploid plants show a remarkable polymorphism in that apparently homologous chromosomes are frequently different in morphology *

---

* The process of detection of chromosome homology is frequently one of elimination in that quite often one has to accept the two chromosomes left over after pairing up all the others as being homologous. Naturally an error at one stage produces an error in all subsequent matchings.

3. Tetraploid plants do not appear to have such a polymorphism. Whether or not this apparent lack of polymorphism is caused by the greater number of pairing possibilities permitting the chromosomes to be more easily forced into a paired karyotype than in diploids is not fully known. Certain chromosomes, in particular Group 4 chromosomes (see Table A , page 18) are most certainly disomic.

4. The chromosomes can be grouped for convenience into five groups according to size, centromere positions and presence of satellites. The demarcation between these groups is often indistinct and is based upon observations made from a few cells which show the morphological differences more distinctly. Table, A, page 18, shows the various details of the groupings.

5. Secondary constrictions appear to demonstrate heterozygosity in that only one of a particular sort of secondary constriction ever appears in a cell, the other homologous chromosome not exhibiting it. This situation is similar to that recently detected in human chromosomes, Beutler (1963), Palmer and Funderburks (1965)*

---

* It is thought that chromatin areas bearing secondary constrictions are late replicating and have other similarities to genetically inactive heterochromatin. This suggests the possibility of chromosomal inactivation in these regions which could be important in terms of genetical adaptation of cells rather than organisms to variations in environment. Heterochromatin bodies are not obviously apparent in resting-state nuclei of Chrysanthemum leucanthemum L. Frequently darker staining clumps of chromatin can be seen but whether these should be interpreted as heterochromatin or simply aggregations of eu-chromatin is not known, although absence of chromatin clumps in some cells makes the latter alternative more likely.

TABLE A

Morphological groupings of somatic chromosomes

	Polyploid	Position centromere	Size chromosome	No. chromosome	Satellited?
Group 1	2N	median	3.5–8 µ	4–6	None
Group 1	4N	"	"	6–10	"
Group 2	2N	submedian	7–8 µ	0–2	None
Group 2	4N	"	"	2–6	"
Group 3	2N	submedian to subterminal	5–6.5 µ	6–10	Two to four
	4N	"	"	8–14	"
Group 4	2N	subterminal	4.5 – 5.5 µ	2–4	All
Group 4	4N	"	3.5 – 4.5 µ	4	"
Group 5	2N	submedian	3.5 – 4.5 µ	2	None
Group 5	4N	"	3.5 – 4.0 µ	2–4	?

Meiotic chromosome variation

Meiotic chromosomal analyses were carried out on anthers using the techniques described in Appendix III.

The main aims of the investigation were as follows:

1. An analysis of chromosome pairing of natural and hybrid plants as an indicator of genomic similarities and dissimilarities. Related to this is an analysis of chiasma frequency and position, in order to discover the basis of differences in pairing behaviour other than the genomic similarities mentioned above.

2. To investigate the results of meiosis in terms of production of viable gametes, especially in polyploids and hybrids.

Diploid meiosis

Meiosis in all diploid plants examined was extremely regular with the production of ring and rod bivalents. There was no evidence of translocations, inversions or the production of univalents. The most common analysable stages were diakinesis, metaphase I and telophase I. Anaphase I preparations were rarely encountered.

Pachytene studies showed that there was complete end to end pairing of homologues. Chiasmata were normally completely terminalised by metaphase (fig. 12, page 20) and almost so by diakinesis (fig. 11, page 20). No instance of more than one chiasma per chromosome arm was observed. Regular telophase 1 configurations (fig. 13, page 20) indicated that disjunction during anaphase 1 was normal and it was assumed that all the diploid plants examined would have produced viable gametes. This assumption was strengthened by examination of stained pollen grains, which showed 95% or more of the grains to be fertile. The reasoning and methodology of the technique used for this are given in Appendix III An analysis of chiasma frequency in plants from different populations was made using diakinesis as the stage of division examined. The results are shown in Table B , page 21.

Dowrick, 1952, found that the average chiasma frequency for diploid plants in the genus Chrysanthemum was 1.50. In the

Fig. 11. Diploid diakinesis

Fig. 12. Diploid metaphase 1.

Fig. 13. Diploid telophase 1 showing products of normal disjunction

TABLE B.

Chiasma Frequency in Diploids

Plant No.	Origin of Material	Average No. Chiasmata per bivalent	Average No. Chiasmata per cell.
1	Loggerheads, Flintshire	1.46	13.13
2	" "	1.56	14.03
3	" "	1.51	13.60
1	Aberdovey, Merionethshire	1.67	15.02
2	" "	1.62	14.56
3	" "	1.59	14.30
1	Bearpark, County Durham	1.66	14.93
2	" "	1.70	15.30
3	" "	1.72	15.48
1	Vallee d'Astos, C. Pyrenees	1.64	14.75
2	" "	1.69	15.20
3	" "	1.61	14.48
1	Malham, Yorkshire	1.55	13.95
2	" "	1.61	14.48
3	" "	1.60	14.40

Chrysanthemum leucanthemum L. plants examined here, the chiasma frequency as shown in the Table above, ranged from below 1.5 to over 1.70 per bivalent. As the plants were all grown under similar environmental conditions, it seems most likely that such differences are genotypic. A difference of 0.05 chiasmata per cell was significantly different at the 5% level. Wide differences observed in chiasma frequency of plants grown from seed taken from the same capitulum indicate segregation of the genes concerned and/or influx of differing genes by outbreeding.

Triploid Meiosis

The meiotic behaviour of triploid plants produced by hybridisation (see page 83) was examined and the following facts were discovered. Meiosis, as examined at metaphase 1, was typical of an autotriploid, with the production of univalents, bivalents and trivalents in varying proportions, Figs. 15 & 16, page 23. The presence of unpaired univalents could be seen in pachytene preparations, Fig 14, page 23, as single threads.

Most of the trivalents were orientated in a convergent fashion with a rod of three as the most frequent configuration. 'Frying pan' and 'Y' configurations involving multiple chiasmata were found fairly frequently. This situation is different to that found in diploids and tetraploids as discussed later. Few instances of linear or indifferent orientation were seen with the result that few chromosomes involved in multivalent formation were left at the equator on disjunction of the trivalents. However, univalents were often left. (Fig. 21, page 27).

Fig. 18, page 25 shows the frequency of trivalents from seven plants plotted against the chiasma frequency. There appears to be a similar relationship for all plants, the correlation coefficient of x upon y being 0.92, significant at the 1% level. The trivalent frequency for the most right hand plant in Fig. 18 has been modified. This plant was an aneuploid containing 25 chromosomes instead of the normal 27.

Fig. 14. Triploid pachytene.
Unpaired univalent indicated
by arrow.

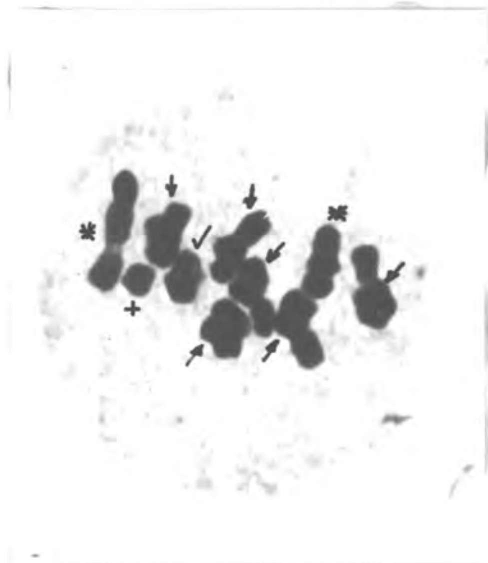


Fig. 15. Triploid metaphase I
→ 6 Frying pan trivalents
✱ 2 linear rod trivalents
✓ 1 ring bivalent
+ 1 univalent

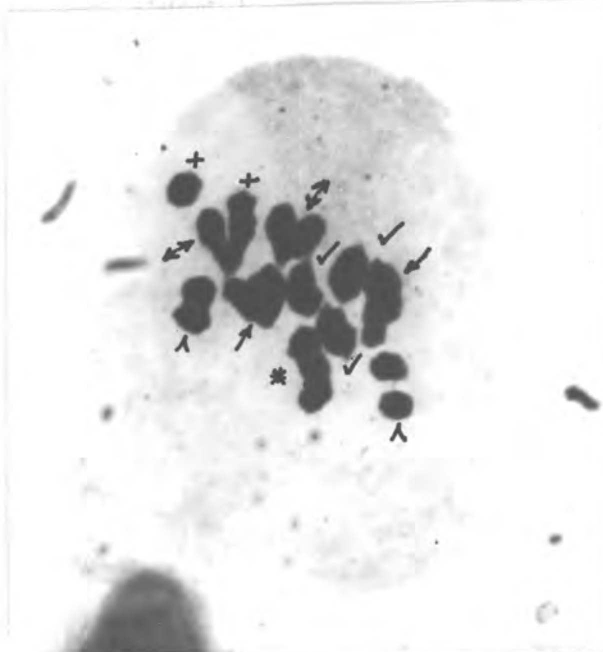


Fig. 16. Triploid metaphase 1.
→ 2 frying pan trivalent
↔ 2 convergent rod trivalents
✱ 1 linear rod trivalent
✓ 3 ring bivalents
+ 2 univalents
∧ 2 univalents showing
precocious division

Fig. 17. Triploid telophase 1
Unequal disjunction resulted
in 13 chromosomes at one pole
and 14 at the other

N.B. 25 chromosome aneuploid

The assumption was made that the two missing chromosomes were non-homologous on a maximum likelihood hypothesis. This being so, then such a plant could only be exhibiting seven-ninths of its potential trivalent production and the observed frequency has been changed to the expected. The modified value falls conveniently onto the x-y axis of the other plants, a fact which reinforces the assumptions made. Although the plants are derived from three different hybridisations involving plants from various parts of Europe, they all show the same degree of chromosomal homology as evidenced by the trivalent-chiasma relationship - an important point which will be discussed later.*

Six hybrids synthesised from an Italian tetraploid and British diploid, although exhibiting normal triploid metaphase pairings had a curious nuclear asynchrony at prophasic stages in which, while one part of the nucleus could be passing through pachytene, other parts of the same nucleus were still at leptotene, Fig. 20, page 27. As only a proportion of P.M.C's examined at prophase exhibited this phenomenon, micro-environmental differences within the stamens must have either induced or suppressed the asynchrony. This phenomenon

---

* Metaphase configurations were used in the analysis and the 'lumpy' appearance of some cells has probably led to an underestimate of trivalent frequency, some 'frying pan' trivalents being scored as separate bivalents and univalents. This error should equal out for all samples.

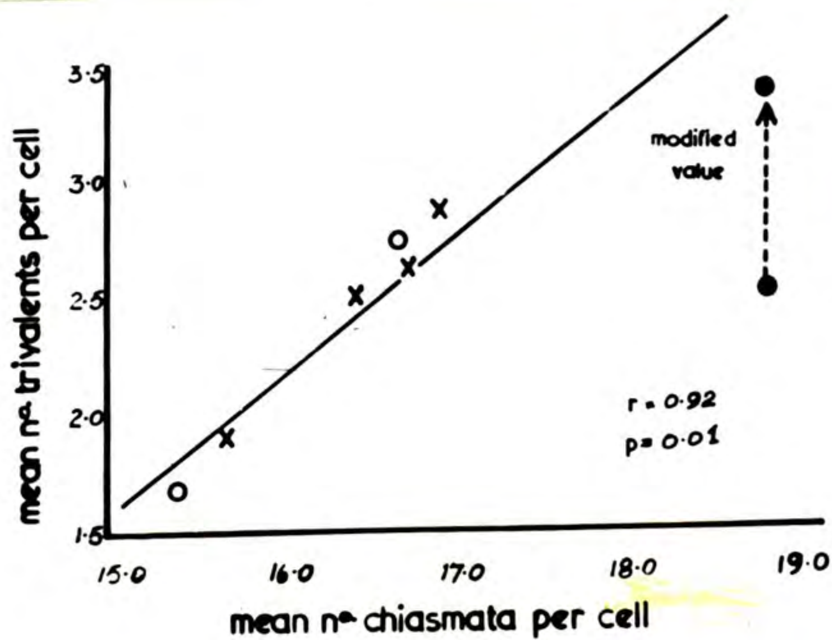


Fig. 18.

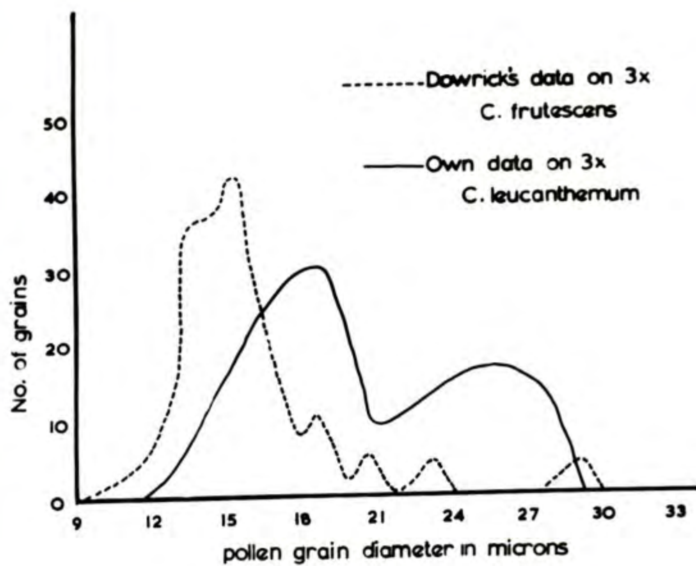


Fig. 19.

was probably caused by a developmental unbalance in the hybrids concerned. The other two types of hybrid did not show such an unbalance.

After examining triploid Chrysanthemum frutescens L. Dowrick (1952) suggested on the basis of pollen grain size measurements that there was production of grains with the haploid chromosome complement and elimination of others with numbers differing from this. The size range of our measurements of triploid plant pollen grains correspond well with Dowrick's data, Fig. 19, page 25, but the distribution and interpretation of the data do not. Examination of telophase 11, Fig. 22, page 27, showed that the pollen grains were likely to contain chromosomes in a frequency which approximates to a binomial distribution. The sample size was insufficient to justify a test of significance for the distribution.

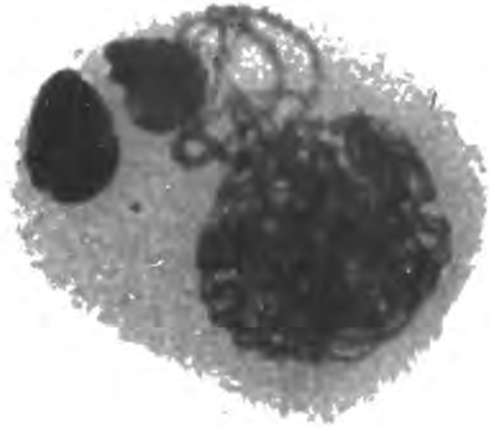
The assumption that pollen grain size is directly proportional to chromosome content is not applicable in this instance since the smaller grain sizes are much smaller than would be expected from a normal haploid situation, as in diploids for example. The apparent skew of the distribution giving a predominance of very small grains possibly results from some hybrid developmental process and is not a direct result of chromosomal content.*

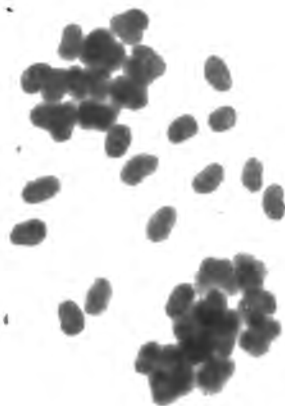
The presence of micronuclei during the tetrad stage of meiosis

---

* The small grains were not empty and stained up in acetocarmine.

Fig. 20.
Asynchronous meiotic
prophase in a
hybrid triploid





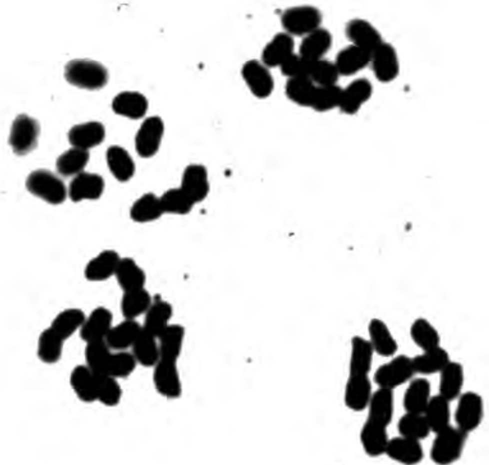


Fig. 21. Late anaphase 1 in a triploid showing lagging univalents which have divided precociously.

Fig. 22. Triploid telophase 11 showing an aneuploid distribution of chromosomes i.e. 15 + 15 + 12 + 12

indicates that some univalents are probably being eliminated by being left on the plate at metaphase I and II. That some univalents must pass to the poles is obvious from the numerically balanced content of most telophase nuclei examined.*

Attempts were made to induce pollen grain division by germination under different substrate, temperature and light conditions, but all failed. This was tried not only for triploid grains, but also for diploid and tetraploid ones.

Dowrick (1952) says that in Chrysanthemum frutescens L. the univalents are lost at both meiotic divisions and this could partially account for his curious explanation of his grain size distribution. At any rate, it would still be necessary to invoke some form of unidirectional elimination of univalents and selection for haploid grains. As regards the small peaks to the right of the main hump, on the numbers of grains involved, these could well be variations in the tail of the main peak or indeed, as Dowrick suggested, products of higher numbers of chromosomes. In fig. 23, page 29, is the distribution of cell classes containing different numbers of trivalents. The distribution is not significantly different from a binomial

---

* In the examination of P.M.C's in which all the chromosomes could be counted at telephase II, frequently the sum of the nuclear contents came to 54. In addition, no chromosomes were obviously left lying between the groups and hence it would seem reasonable to expect all the chromosomes to be included into the pollen grains. However, only the examination of pollen mitosis would verify this point.

Fig. 23.

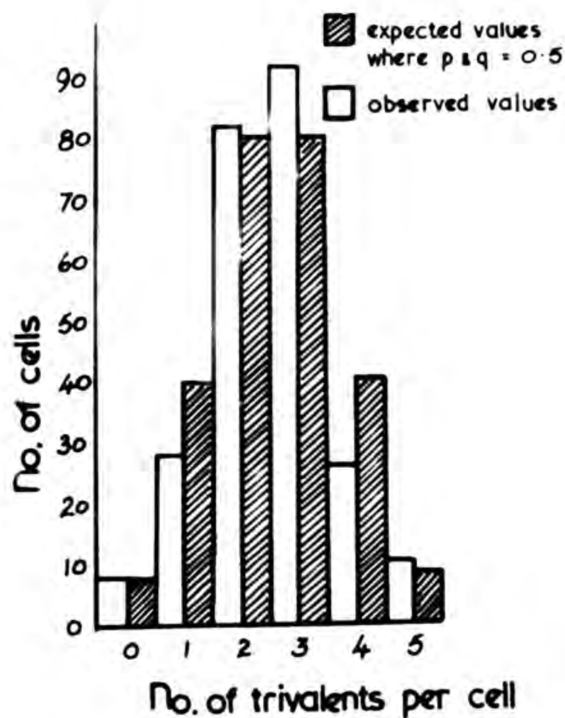


Fig. 24. Diakinesis in a natural tetraploid
1 ring quadrivalent
1 rod quadrivalent
3 ring bivalents
11 rod bivalents

Fig. 25. Diakinesis in an induced tetraploid.
2 ring quadrivalents
1 rod quadrivalent
5 rod bivalents
7 ring bivalents

distribution which indicates random pairing and chiasma formation between homologues.

Tetraploid meiosis

Examination of tetraploid meiosis showed the following points:-

1. Multivalent associations were regularly found in between 65% and 90% of P.M.C's examined at diakinesis and metaphase I. These were usually ring or rod quadrivalents, figs. 24 & 25, page 29 and figs. 26, 27 & 28, page 31 . They were regarded as homologous chromosome associations and not due to interchange heterozygotes for the following reasons:-

(a) Disjunction was always regular, (fig. 29, page 31) and only some of the quadrivalents showed the formation necessary for balanced disjunction in translocation associations.

(b) Up to four quadrivalent associations have been seen in one cell. To account for this either a high degree of chromosome homology or else several separate translocations or both must occur. Since no associations involving more than four chromosomes have been seen, the latter alternative seems unlikely.

(c) The high degree of pollen fertility, normally over 95%, is a far higher value than one would expect in plants in which all the multivalents were not orientated necessary for balanced disjunction.

[Table C , page 32 , gives the quadrivalent frequencies for a range of wild tetraploids and artificially induced autotetraploids. The

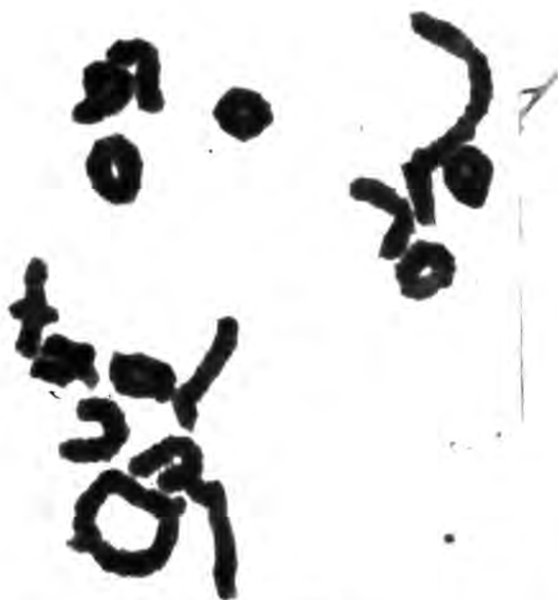


Fig. 26. Diakinesis in natural tetraploid
1 ring quadrivalent
1 rod quadrivalent
6 ring bivalents
8 rod bivalents

Fig. 27. Metaphase 1 in a natural tetraploid
1 ring quadrivalent with divergent orientation
1 rod quadrivalent with divergent orientation

Fig. 28. Metaphase 1 in an induced tetraploid
1 ring quadrivalent with linear orientation ✓
1 rod quadrivalent with linear orientation →

Fig. 29. Telophase 1 in natural tetraploid showing products of normal disjunction

quadrivalent frequencies are scored as the mean number of quadrivalents per cell.

TABLE C.

Quadrivalent frequencies in different tetraploids

Number of cells analysed	Quadrivalents per cell	Source of Material
35	1.41	Buxton 6, Derbyshire
30	1.63	A2/3 Autotetraploid
30	1.37	A2/12 "
80	1.33	A2/16 "
50	1.66	Malham 1
30	1.58	Fen End, Shropshire
50	1.48	Central Bohemia
30	.78	Zagreb, Yugoslavia
30	.67	Southern Czechoslovakia

It is obvious that the last two continental plants have much lower frequencies of quadrivalents per cell than do the rest of the plants. The difference between the quadrivalent frequencies for the Zagreb and A2/16 plants was tested and found to be significant. This difference may be associated with taxonomic differences since the plants have some morphological differences to the British tetraploids.

2. Pachytene pairing is normal with apparently complete pairing along the whole chromosomes. The extreme length and number of the chromosome threads makes a full analysis of 'incipient multivalent' pairing impossible. The centromeres appear to be fully paired at pachytene.

3. The mean chiasma frequency per plant varies between 23 and 28.

There seems to be a relationship between the mean frequency of quadrivalents produced and the mean chiasma frequency of the plant concerned. See fig. 30, page 34 . For the nine plants used the correlation between these two parameters was -062, p being =0.05. *
What the significance of a negative correlation between these parameters is, seems difficult to elucidate and an examination of the factors controlling multivalent formation will be more conveniently carried out in the discussion. It must be pointed out that this value is only just significant and more data on this point are required. It does, however, raise ground for speculation.

In Table D is shown the chiasma frequencies for a range of diploid, and tetraploid plants. It can be seen that generally the tetraploids have a chiasma frequency per chromosome somewhat less than that of the diploids.**

** Triploids have not been included because of the possible error of having underscored 'frying pan' configurations.

---

* It was discovered that fifteen cells were sufficient to accurately define the mean chiasma frequency for a plant. However, thirty or more <u>randomly</u> selected cells were necessary to define the mean quadrivalent frequency, since when only well spread preparations were analysed, these contained a higher frequency of multivalents than did a random selection of cells. The size and characteristic shape of quadrivalents, made them easy to identify even in poorly spread cells.

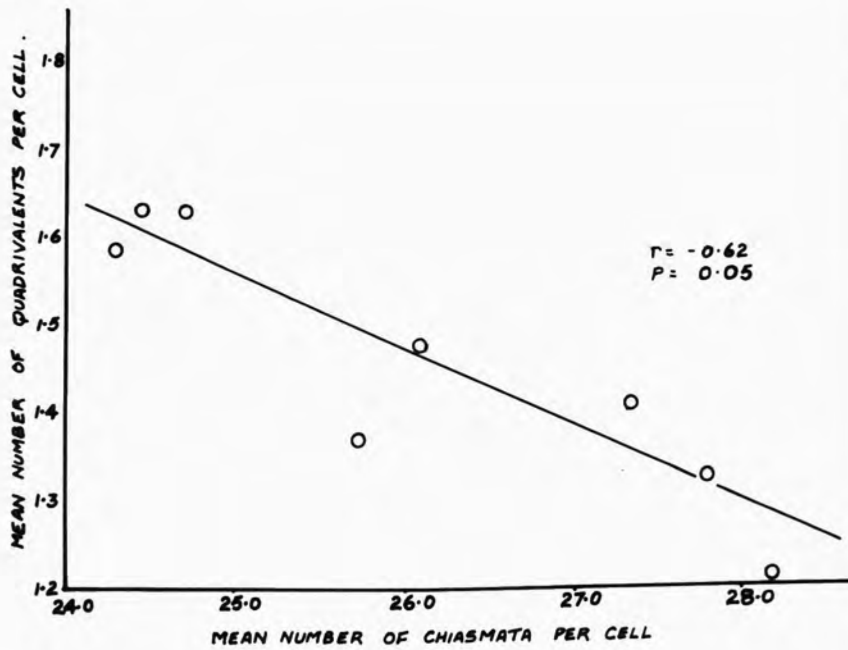


Fig. 30.

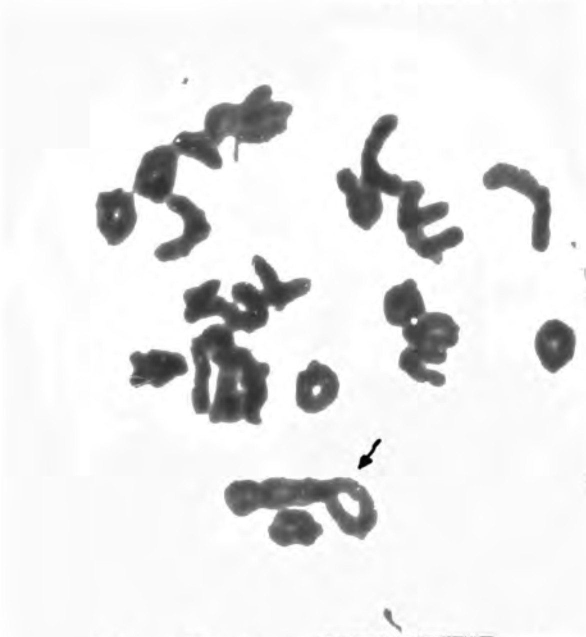


Fig. 33. Hexaploid diakinesis. Ring hexavalent indicated by arrow.

Fig. 34. Late tetraploid diplotene, chiasmata are fairly terminal. Ring quadrivalent indicated by arrow.

TABLE D

Chiasma frequency for diploids and tetraploids

No. cells analysed	Chromosome count	Mean No. Chiasmata/ cell	Mean No. Chiasmata/ chromosome	
50	2n = 18	14.50	.806	Bearpark 7, Co. Durham
50	2n = 18	14.85	.825	Bearpark 10, Co. Durham
50	2n = 18	14.60	.812	Malham Moor 6, W.Yorks
75	2n = 18	13.20	.735	Loggerheads 6, Flints.
50	2n = 18	14.30	.795	Festiniog, Caerns.
50	2n = 18	14.20	.789	Aberdovey 9, Merioneth.
35	2n = 36	27.32	.758	Buxton 6, Derbyshire
30	2n = 36	24.73	.686	A2/3) artificial auto-
30	2n = 36	25.74	.716	A2/13) tetraploids pro-
80	2n = 36	27.78	.772	A2/16) duced from material from Loggerheads, Flints.
30	2n = 36	24.27	.675	Fen End, Shropshire
50	2n = 36	26.19	.728	Central Bohemia
30	2n = 36	22.85	.636	Zagreb, Yugoslavia
30	2n = 36	27.94	.777	Southern Czechoslavakia
50	2n = 36	24.41	.679	Malham, Yorks.

4. No micronuclei could be found in most plants examined but some were detected in the artificial autotetraploid A2/16 and this plant was subjected to a thorough analysis. Out of 300 pollen tetrads examined, 27 contained micronuclei, several being almost as large as the normal nuclei but most being extremely small. An analysis of 50 P.M.C's at late anaphase - early telophase revealed no lagging chromosomes and hence apparently normal disjunction. The reason for normal disjunction of quadrivalent chromosomes is because chiasmata are terminal and relatively few in number, thus allowing for the efficient and regular separation of the chromosomes. As most of the

cells were examined at diakinesis, it is not known what proportion of quadrivalents were orientated in a linear or convergent fashion. At metaphase I as in fig. 27, page 31, some of the quadrivalents were certainly convergent. Instead of the normal distribution of 18 bivalents at each pole, three cells had 17 bivalents at one pole and 19 at the other. This must be a possible source of unbalanced gametes which could give rise to aneuploid plants.

Examination of cells at anaphase II - telophase II revealed the source of the micronuclei. A small proportion of the cells at this stage had univalents left lying on the metaphase plates. Slightly later stages showed that these laggards were separated from the normal nuclei on formation of the tetrad cell walls.

5. With the exception of a higher production of micronuclei indicating a slight meiotic unbalance, the eight artificially induced autotetraploids examined were identical in chromosome pairing and chiasma production to all natural tetraploids examined - a fact of considerable importance.

6. There was no evidence of interstitial chiasmata or configurations resulting from multiple chiasmata. This leads to the conclusion that some mechanism is working which is limiting the production of chiasmata to one per chromosome arm.

7. There was a low frequency of trivalents and univalents, a fact which adds to the high fertility of tetraploid plants. The possible reasons for such low frequencies will be discussed later.

Hexaploid meiosis

The meiosis of several hexaploid plants of continental origin showed the following points:-

1. Multivalents were produced and these were generally hexavalents or quadrivalents, but rarely both (fig. 33, page 34). A maximum of two quadrivalents was found in any one cell. It could not be ascertained whether the multivalent production was caused by chromosomal homology or by interchange heterozygosity.
2. All the remaining configurations were rod or ring bivalents.
3. Telophase I counts indicated that normal disjunction occured at anaphase I.
4. Chiasmata were terminal at metaphase I, and there was no evidence of interstitial chiasmata or configurations resulting from multiple chiasmata.

Discussion

It is apparent that somatic chromosome morphology does not give a reliable insight to genome identity. This is due in part to the variability with which the chromosomes present themselves under the microscope and also due to slight genuine changes in morphology. Recognition of similar patterns of variation between populations is consequently a hazardous and time-consuming process. The evidence suggests that chromosome similarities exist between diploid populations of British plants and between tetraploid populations. There is some small variation in chromosome morphology between diploid and tetraploid

populations indicating at least partial genome differences. The large number of morphologically identical chromosomes present in both polyploid levels confirm that similar chromosome morphology does not imply chromosomal homology - usually a basic tenet of karyotype analysis. Heteromorphic pairing in diploid plants probably results from outbreeding between populations with slight chromosomal differences, of a degree which is insufficient to affect the efficiency of forming chiasmate associations. Unless selected against, cytological heterozygosity of this nature is likely to remain within a population since chiasmata are confined to the ends of the chromosomes. Thus, structural recombination only occurs near the end of the chromosomes and leaves a long pericentric length of chromatin unchanged. Such linkage groups can accumulate both gene and chromosome mutations and contribute immensely to the retention of successful genetical sequences within populations without danger of the sequences being split up by recombination. This is a situation analogous to that postulated in Anthoxanthum odoratum, Jones, K. (1964).

The presence of some certain disomic chromosomes in tetraploid populations reflects either establishment of homozygosity by autopolyploidy or hybridisation at some stage in the evolution of the populations concerned. It is tempting to suggest the former course when Group 4 chromosome variation is examined. See Appendix V page168 . The variation of these chromosomes is identical to that expected if the tetraploids concerned had arisen from a population heterozygous for the

marker chromosome.

The use of chromosome pairing behaviour, and frequency analysis of various chiasmate associations for detecting genomic differences between closely related taxa, are hazardous tasks, since observed differences may be an expression of gene differences rather than genomic, a point appreciated by Jones and Borrill (1962). The control of pairing behaviour and chiasma formation by single or closely linked genes has now been well established, (Riley, Chapman and Kimber (1959), Rees (1961), and unless a pattern of relationship between chiasma frequency and frequency of different chiasmate associations can be established, then meiotic differences between organisms are open to misinterpretation as being due to genomic differences and not genic or vice versa.

Quadrivalent formation in the tetraploids indicates a higher degree of chromosomal homology than would be expected from plants of completely allopolyploid origin. However, when the frequencies of quadrivalents produced are compared to the values obtained in species of known autoploid origin by other workers, there seems to be a considerable reduction in the observed values (see Table E , page 41). The exception to the other values is that of Oksala's, (1952) who noted 27.2% of the chromosomes occured as quadrivalents in spontaneously produced spermatocytes in a dragon-fly species. The immediate conclusion is to regard Chrysanthemum leucanthemum L. as a segmental allotetraploid species, (Stebbins (1950), exhibiting partial genome

homology. The information from newly synthesised autotetraploids, however, does not confirm this idea and Fig. 32, page 42, shows the frequency distribution of cells containing various numbers of quadrivalents from eight artificial tetraploids, natural tetraploids and a single artificial tetraploid for which there was sufficient data. The distributions are not significantly different from each other or a binomial distribution where p and q are 0.27 and 0.73, respectively. McCollum (1958) regards a binomial distribution as being indicative of a situation in which the probabilities of quadrivalent formation are the same for each set of four chromosomes in a cell and for each cell in the organism concerned.

Hall (1955) using data taken from the literature demonstrated that chromosome pairing in hybrids between closely related species is uniform from one set of chromosomes to another but in hybrids of unrelated species is not uniform and therefore, does not correspond to a binomial distribution. On this basis the Chrysanthemum data would fit a situation where there is non hybridity, and hence an expectation for quadrivalent formation by chromosome sets. Some factor, other than lack of homology, is limiting the production of quadrivalents and forcing the quadrivalent frequency distribution towards the zero end of its spectrum.

The similarity of pairing behaviour in natural and induced tetraploid Chrysanthemum leucanthemum L. and the apparent cytological differences between these plants and other autotetraploid-like species warrants an examination of the factors involved in chromosome pairing in an autotetraploid.

TABLE E.

Frequency of quadrivalents in a number of induced tetraploids of different species

Species	Author	Year	Mean No. quadrivalents per cell	% of chromosome complement occurring as quadrivalents	Chromosome number
Avena strigosa - Ottawa	Morrison & Rajhathy	1960	4.4	62.8%	2n = 28
Avena strigosa - Japan	"	1960	4.0	57.1%	2n = 28
Hordeum vulgare	Tsuchiya	1957	3.0	43.0%	2n = 28
Secale cereale	Morrison	1956	3.9	56.7%	2n = 28
" "	Müntzing	1951	3.9	56.7%	2n = 28
Hordeum bulbosum	Morrison & Rajhathy	1960	4.0	57.1%	2n = 28
" "	Davies	1958	4.7	67.2%	2n = 28
Dactylis glomerata	McCollum	1958	3.0	43.0%	2n = 28
	Jones	1958	3.5	48.6%	2n = 28
Asparagus officinalis	Morrison & Rajhathy	1960	6.9	69.0%	2n = 40
Lycopersicum esculentum	Morrison & Rajhathy	1960	7.0	63.0%	2n = 48
Aeschnea subarctica elisabethae	Oksala	1952		27.4%	2n = 54
Chrysanthemum leucanthemum L.	Present work		1.3 - 1.7	15% - 20%	2n = 36

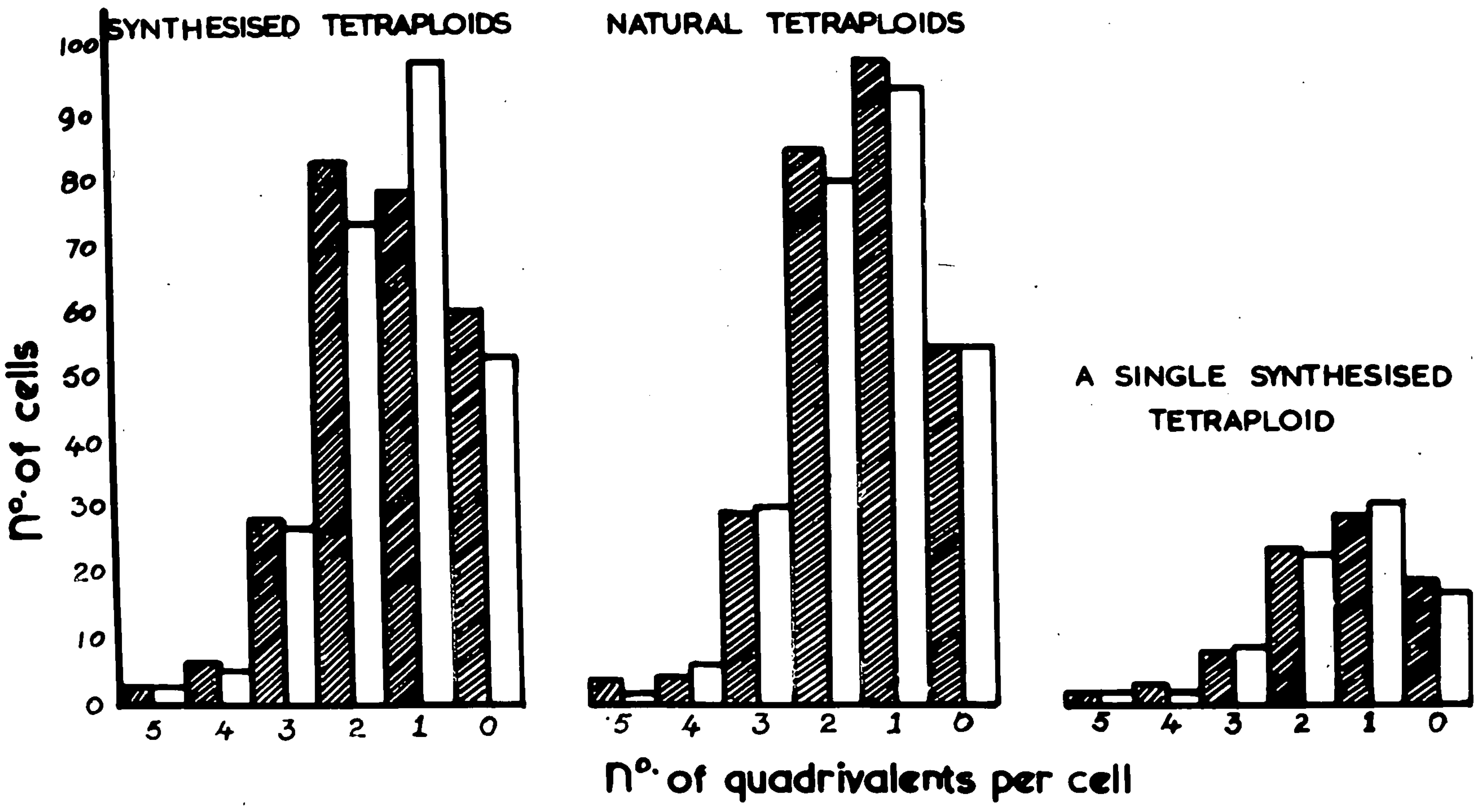


fig.32

The formation of a quadrivalent rather than two bivalents, a trivalent and one univalent or four univalents from a set of four homologous or partially homologous chromosomes is dependent on (a) at least one change of pairing partners during pachytene by at least one of the chromosomes (b) the formation of sufficient chiasmata to maintain the pairing configurations. The following generalisations can be made about the pairing processes of Chrysanthemum leucanthemum L. chromosomes. (a) The chromosomes are all approximately the same size and have more or less metacentric centromeres. This means on face value that probably each chromosome arm is just as likely to pair and form chiasmata as any other, unlike the situation found by John and Henderson (1962) in tetraploid spermatocytes of Schistocerca paranensis, where the longer chromosomes formed more chiasmata and hence quadrivalents than did the shorter ones. (b) The lack of interstitial chiasmata or configurations resulting from multiple chiasmata implies that a mechanism limiting chiasmata to one per chromosome arm is operating. In terms of the pairing block concept, (Darlington and Mather, (1932), Darlington (1937), Klindstedt (1937),) this might be interpreted as resulting from the initiation of pairing at only a single point in any one chromosome arm, i.e. each arm forms a single potential pairing block. Oksala (1952) and McCollum (1958) have postulated a similar mechanism in the tetraploid organisms that they were studying. In Chrysanthemum leucanthemum L. diplotene observations show that the

chiasmata are first evident close to the chromosome ends, in most instances situated about two-thirds of the way along the chromosome arm from the centromere. See fig. 34, page 34. It is interesting to note that the one chiasma per arm mechanism breaks down in the triploid hybrids as evidenced by 'frying pan' and 'y' trivalents. The precise correspondence between the number of chiasmata formed and the number of linked chromosome arms means that theoretical models of chromosome pairing and chiasma formation can be easily developed and used to compare observed values for chromosome configurations with the expected. Changing the mechanics of the models employed to derive a better fit to the observed data can be helpful in elucidating the processes involved in meiotic division.

Durrant (1960) has developed a mathematical model for comparing the chromosome association frequencies observed against the frequency of associations expected, assuming that they arise from the random formation of chiasmata between each set of four homologous chromosomes in an auto-tetraploid. From four chromosomes the five possible types of association for any given chiasma frequency P can be calculated from the formulae given. As Durrant (1960) points out when calculating the expected chromosome associations for any given organisms, the value of P cannot be derived from the observed mean chiasma frequency and some assessment of the variance of P has to be taken into account. In Table F , page 45 , are the expected association frequencies calculated for various values of P.

TABLE F

Expected association frequencies calculated for various values of P.

Chiasmata per cell	P	1(2) + 2(1)	2(2)	1(3) + 1(1)	1(IV)
33	3.66	.89%	4.8%	31.18%	63.13%
31	3.44	1.26%	5.5%	34.25%	58.92%
29	3.22	1.87%	6.84%	39.14%	52.14%
27	3.00	2.78%	8.33%	44.44%	45.55%
26	2.88	3.44%	9.20%	47.39%	39.96%
24	2.66	5.10%	11.01%	53.02%	30.87%
22	2.44	7.57%	12.96%	58.51%	20.96%
20	2.22	11.23%	14.92%	64.55%	9.30%

From this table the expected association frequencies were calculated for one induced and one natural tetraploid plant and compared with the observed frequencies. The distribution of P in Chrysanthemum leucanthemum L. is unimodal and fits a normal distribution.

Malham tetraploid 1

	1(2) + 2(1)	2(2)	1(3) + 1(1)	1(4)
Expected	5.3%	10.8%	49.5%	34.4%
Observed	1%	80.7%	.1%	18.2%

Flintshire autotetraploid 16

	1(2) + 2(1)	2(2)	1(3) + 1(1)	1(4)
Expected	2.8%	8.9%	40.0%	48.0%
Observed	0%	85.1%	.1%	14.8%

The differences between the observed and expected values for Durrant's model are highly significant. John and Henderson, (1962)

have criticised this model on the basis of the high trivalent and univalent frequency expected by it. Most published data (see Table H page 47) have a negligible frequency of trivalents and univalents and a higher frequency of bivalents and quadrivalents than would be expected according to Durrant's model. They proposed an alternative model based not on random, but partial obligatory pairing between homologous chromosomes. Their diagram outlining this model is reproduced in fig. 35, page 48. Independently, a similar model to that of John and Henderson, was developed at Durham. It had two alternative pairing patterns to it, version two being identical to that of John and Henderson, and version 1, differing in that the second chiasma to be inserted into any homologous set could occupy any one of the four remaining possible positions on a one chiasma per chromosome arm basis. This is shown in fig. 36, page 48. The third chiasma to be inserted is obliged to occupy one of two positions and the fourth has no alternative. The two alternatives were reproduced in an Elliot 803 computer by simulating meiotic prophase using 9 homologous sets of chromosomes and observed distributions of P. Fifty metaphase I cells were analysed, this sample size being considered large enough to characterise the distribution of P. The computer approach was adopted since the individual processes involved were much easier to conceive in terms of physical rearrangement inside a computer than in mathematical formulae expressing such manipulations. The programmes and data print-outs are given in Appendix VI page 169.

The Table below gives the expected values for the two versions and observed values for the plant from which the P distribution was taken.

TABLE G	Univalents	Bivalents	Trivalents	Quadrivalents
Version 2	5%	45.7%	0%	49.4%
Version 1	6.4%	40.2%	4.9%	48.5%
Observed	.03%	85.1%	.07%	14.8%

The following points arise:-

(a) Neither version fits the observed data well but both would approximate to published data in other species.

(b) Version 2, similar to that of John and Henderson (1962), will always give a zero value for trivalent frequency, a point which is not normally realised in nature where a low but consistent production is usual.

Version 1 appears to fit other data better for all chromosome classes. In the table below this model is compared to McCollum's (1958) data on the induced tetraploids of subspecies lusitanica, juncinella and ibizensis of Dactylis glomerata.

TABLE H	Univalents	Bivalents	Trivalents	Quadrivalents
Version 1	6.4%	40.2%	4.9%	49.4%
D. glomerata s.sp. lusitanica	2.5%	39.2%	2.8%	55.5%
S.sp. juncinella	2.75%	42.7%	2.0%	52.6%
S. sp. ibizensis	1.1%	45.7%	1.1%	52.1%

Considering that the expected values have been worked out using a different distribution of P from the one in Dactylis glomerata, the correspondence

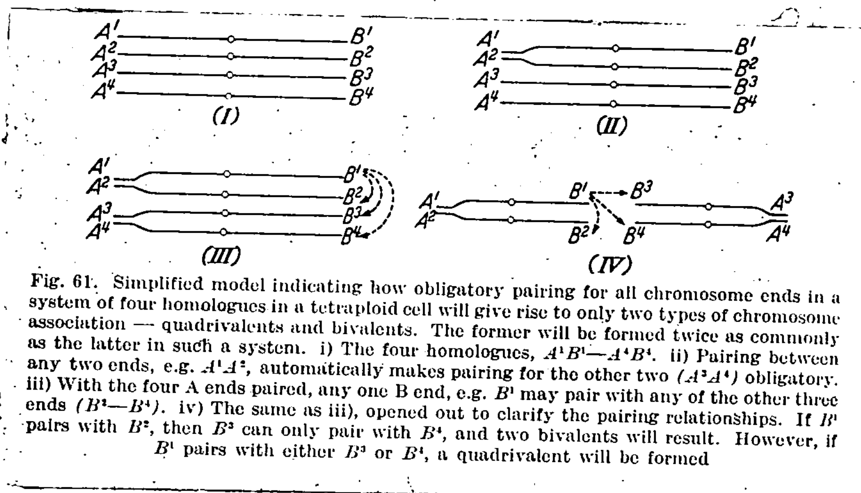


Fig. 61. Simplified model indicating how obligatory pairing for all chromosome ends in a system of four homologues in a tetraploid cell will give rise to only two types of chromosome association — quadrivalents and bivalents. The former will be formed twice as commonly as the latter in such a system. i) The four homologues, A^1B^1—A^4B^4. ii) Pairing between any two ends, e.g. A^1A^2, automatically makes pairing for the other two (A^3A^4) obligatory. iii) With the four A ends paired, any one B end, e.g. B^1 may pair with any of the other three ends (B^2—B^4). iv) The same as iii), opened out to clarify the pairing relationships. If B^1 pairs with B^2, then B^3 can only pair with B^4, and two bivalents will result. However, if B^1 pairs with either B^3 or B^4, a quadrivalent will be formed

Fig. 35. Version 2. Chromosome pairing scheme after Henderson and John.

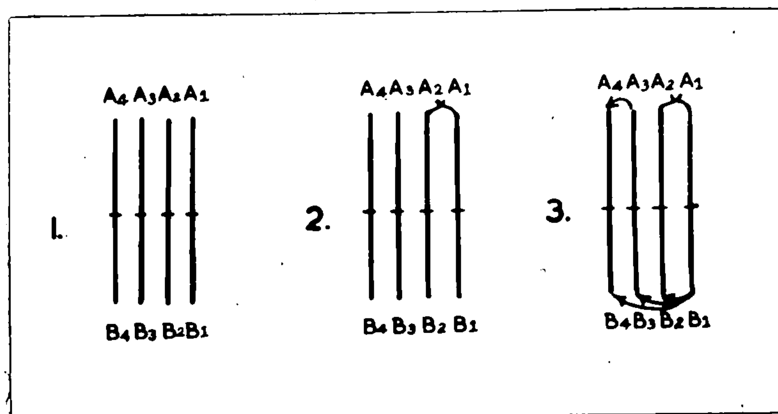


Fig. 36. Version 1. The first chiasma is inserted in A_1-A_2. Subsequent chiasmata can occupy any other possible location as shown in (3)

between the observed and expected is acceptable.

The reduction in quadrivalent and increase in bivalent frequency in Chrysanthemum leucanthemum L. from the expected is more difficult to interpret, but could be explained on the following basis. Slight structural differences between homologous chromosomes in the diploid state could result in preferential pairing upon induction of tetraploidy. It seems reasonable to postulate changes in pairing behaviour rather than in differential chiasma formation. At the tetraploid level, such differences could be maintained by continued outbreeding and mutation within the pericentric linkage groups (page 38). It is not necessary to invoke a concept of evolution towards diploidisation at the tetraploid level since newly induced tetraploids immediately exhibit an identical reduction in quadrivalent frequency. Oksala (1952) has suggested a similar reduction in quadrivalent frequency in the Odonata, but on different grounds. In the diploids of these organisms there is chiasma interference across the centromere resulting in only rod bivalents being formed. In the tetraploid segments of testes exhibiting quadrivalent production and hence chiasmata formation on each side of the centromere, he suggests that there has been a break down of chiasma interference across the centromere due to incomplete pachytene pairing in the centromeric region. Oksala states that where pachytene pairing is complete then normally only one chiasma per chromosome pair is formed, the first one being obligatory and subsequent ones being formed only where centromere pairing is incomplete.

The later case is the only situation under which quadrivalents could arise.

This theory does not apply to Chrysanthemum leucanthemum L. since there is no evidence of chiasma interference across the centromere and pachytene pairing appears to be complete.

The concept of a first obligatory chiasma stated explicitly by Owen (1949) seems to be applicable to most pairing analyses. He says - "the first chiasma to be formed in a chromosome pair, being a necessity for bivalent formation is not on equal footing with chiasmata formed later." When one compares quadrivalent frequency in cells of low chiasma frequency against quadrivalent frequency in cells of high chiasma frequency taken from the same plant then surprisingly there does not appear to be a much higher frequency of quadrivalents in the latter class than in the former, the difference being expressed principally in frequency changes of ring and rod bivalents. Also there is not a higher frequency of univalents and trivalents in the low chiasma class. This implies that all chromosome pairings receive at least one chiasma initially and there could well be competition for the remainder, a point which has been developed by Basak and Jain (1963) in Delphinium.

Pairing in the triploid hybrids shows quite definitely that similar chromatin material exists in both diploid and tetraploid plants. An important fact arising from the examination of triploid pairing is

that identical pairing behaviour is exhibited by three different hybrid types, all involving British diploid and continental tetraploid parents from different localities indicating that genic divergence, evidenced by external morphological differences of the parents, has not been accompanied by differences in pairing properties of the chromosomes concerned. The fact that the only hybrids that could be established involved plants from different parts of Europe is of interest for it may indicate that incompatibility mechanisms exist amongst plants from the same region. Too much emphasis should not be read into this, as it is based on only three successful hybridisations. The cytological instability of triploid hybrids, 862/3 → 462/1 is probably caused by some cytoplasmic unbalance. Rees, (1958) has described a similar case in Scilla where differential chromosome contraction was observed between long and short chromosomes. The differential behaviour of the chromosomes involved in the Chrysanthemum leucanthemum L. hybrids might well result from different responses of the two composite genomes. The evidence from metaphase II counts in triploids indicates that the frequency of pollen types with differing numbers of chromosomes is following a binomial distribution indicating random disjunction, a fact noted by Yanney-Wilson (1959), Avera (1954) Jones and Borrill (1962) and numerous other authors. It seems most likely that only the fertile pollen grains are going to correspond to the euploid numbers and on a binomial distribution the expectancy of these would be negligible. Satina and Blakeslee (1937) working with

triploid Datura stramonium found considerable pollen fertility through the production of far greater numbers of euploid grains than expected. They attributed this to the non random orientation of trivalents at metaphase I. There is no evidence for a similar mechanism in triploid Chrysanthemum leucanthemum L. Q. Kay (personal communication) has successfully backcrossed triploid Tripleurospermum maritimum (a not too distantly related genus) to diploid and tetraploid parents indicating that the triploids have partial fertility. Personal attempts at backcrossing triploids to their parents have all failed but this may be a consequence of an insufficient number of attempts to achieve success. Jones and Borrill (1962) consider the slight triploid fertility of Dactylis glomerata to be of great evolutionary consequence in maintaining a gene flow between diploid and tetraploid levels and in the Iridaceae exemplified by the genus Sysyrynchium, Ingram (personal communication) and Gladiolus, Jones and Bamford (1942), Hamilton (personal communication), hybridisation between polyploid levels has broken down discontinuities between the original parents.

In Chrysanthemum leucanthemum L. gene flow between diploid and tetraploid levels by means of an intermediate hybrid is problematic since triploids have never been discovered in nature. Danielli and Zohary (1961) discovered seven triploids in an examination of 4,000 plants from a population of Dactylis glomerata. It seems likely, in view of the difficulties of hybridising diploid and tetraploid

Chrysanthemum leucanthemum L. that the occurrence of natural triploids is either going to be negative or on a comparable scale of rarity as in Dactylis. Whilst such hybrids may have a small contribution to make to possible gene exchange between ploidy levels, the most probable source of gene flow is by the production of unreduced gametes by diploid plants. Evidence that such an undirectional gene flow could occur is presented elsewhere, Chapter VII, page 131.

Conclusions

The evidence suggests that Chrysanthemum leucanthemum L. is a species in which successful polyploidisation to the tetraploid level has been achieved by simple genome reduplication. It is thought that hybridisation between widely different diploid races has not been a necessary corollary to the origin of tetraploids and that induction of polyploidy has not been a mechanism for regularising meiosis. The diploid plants which gave rise to the tetraploid races were genetically similar to existing diploid stocks. In the sense that the putative diploid stocks involved probably exhibited complete bivalent pairing and fertility, then the tetraploids produced can confidently be regarded in cytological terms as having had an autoploid origin. Genetically, however, the tetraploids are probably not tetrasomic at each locus and hence in terms of gene content should be regarded as segmental allopolyploids, Stebbins (1950). Diagrammatically this is expressed over the page.

A' A	putative diploid ancestor with complete chromosome pairing and fertility.
A'A' AA	segmental allotetraploid exhibiting preferential pairing.

The outbreeding behaviour of the species and the introduction of chromosome variants from different populations has probably obscured the morphological identity of chromosome homologues.

S E C T I O N   II

GEOGRAPHICAL AND ECOLOGICAL VARIATION

IN CHRYSANTHEMUM LEUCANTHEMUM L.

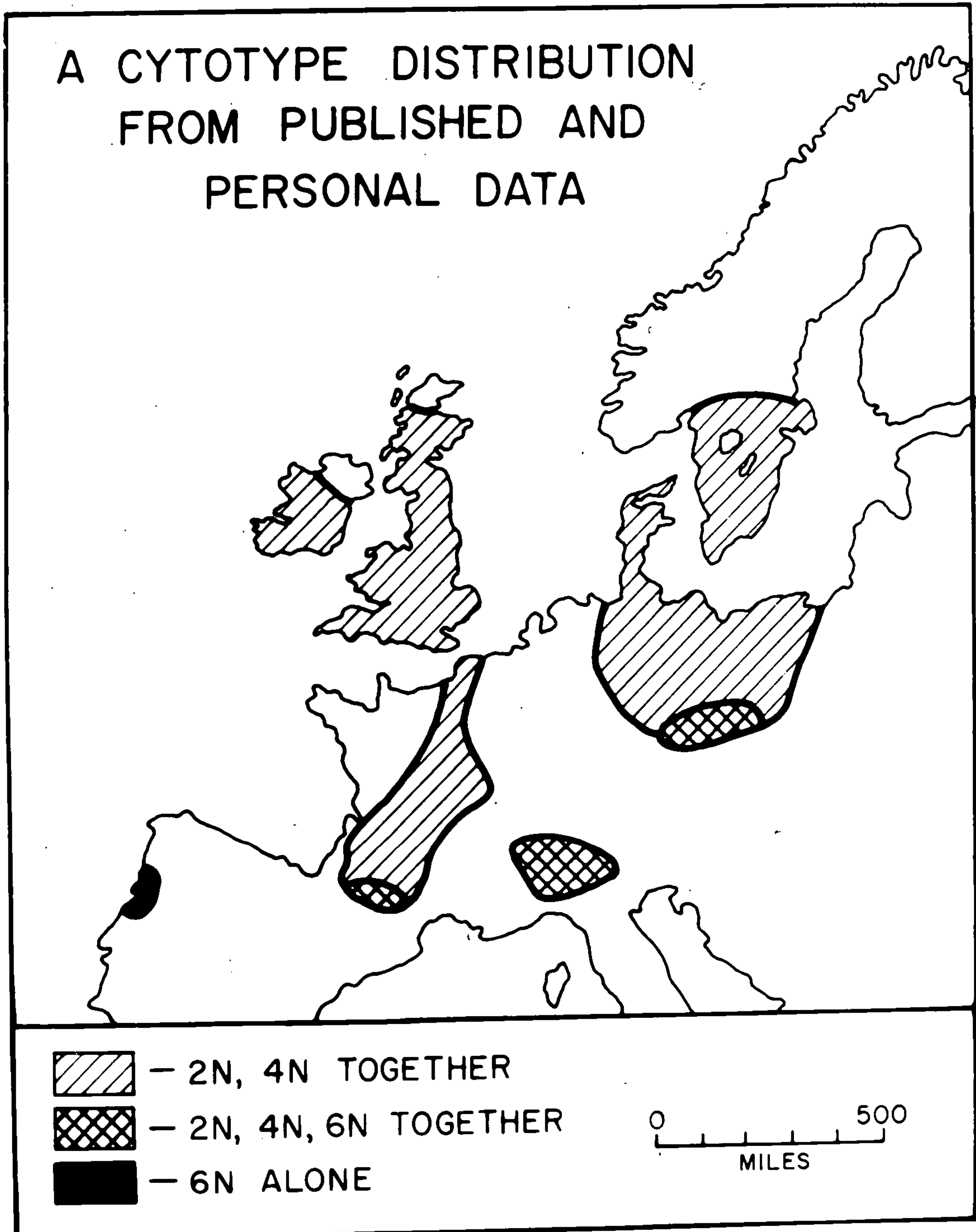
SENSU LATO

GEOGRAPHICAL AND ECOLOGICAL VARIATION IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

Chrysanthemum leucanthemum L. sensu lato has a north temperate distribution and is found on both the American and Eurasian land masses. The southern limits of the aggregate in N. America appear to be the mid-western states where the plants have numerically reached pest proportions, and the farmers there are expected to uproot and burn the plants under threat of fine. In Europe the southern limits are defined by the Mediterranean basin and in Asia by the Pamir-Himalayan mountain chain. The eastern limits of the aggregate are most difficult to ascertain but it certainly stretches as far east as Irkutsk from where the type specimen of C. ircutianum Turcz., probably synonymous with part of Chrysanthemum leucanthemum L., was first described. In both North America and Europe the northern limits appear to be confined within the latitudes 60°N and 70°N respectively. The cytotype distribution map shown on page 56, fig. 37 , is based upon approximately 300 locality counts taken from the literature and our own work, the latter being given in Appendix I , page 134. It can be seen that diploid and tetraploid plants are sympatric over most of Europe with diploid, tetraploids and hexaploids being sympatric in the alpine regions of central Europe. Mulligan's (1959) work shows that diploids and tetraploids are found in North America. Favarger (1959) Baksay (1957), Skalinska et al (1961, 1963, 1964) and Favarger and Villard (1965, 1966), have carried out extensive studies within small geographical regions in central Europe and have shown the following points:-

GEOGRAPHICAL AND ECOLOGICAL VARIATION IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

Chrysanthemum leucanthemum L. sensu lato has a north temperate distribution and is found on both the American and Eurasian land masses. The southern limits of the aggregate in N. America appear to be the mid-western states where the plants have numerically reached pest proportions, and the farmers there are expected to uproot and burn the plants under threat of fine. In Europe the southern limits are defined by the Mediterranean basin and in Asia by the Pamir-Himalayan mountain chain. The eastern limits of the aggregate are most difficult to ascertain but it certainly stretches as far east as Irkutsk from where the type specimen of C. ircutianum Turcz., probably synonymous with part of Chrysanthemum leucanthemum L., was first described. In both North America and Europe the northern limits appear to be confined within the latitudes 60°N and 70°N respectively. The cytotype distribution map shown on page 56, fig. 37 , is based upon approximately 300 locality counts taken from the literature and our own work, the latter being given in Appendix I , page 134. It can be seen that diploid and tetraploid plants are sympatric over most of Europe with diploid, tetraploids and hexaploids being sympatric in the alpine regions of central Europe. Mulligan's (1958) work shows that diploids and tetraploids are found in North America. Favarger (1959) Baksay (1957), Skalinska et al (1961, 1963, 1964) and Favarger and Villard (1965, 1966), have carried out extensive studies within small geographical regions in central Europe and have shown the following points:-


A CYTOTYPE DISTRIBUTION FROM PUBLISHED AND PERSONAL DATA
— 2N, 4N TOGETHER
— 2N, 4N, 6N TOGETHER
— 6N ALONE
0
500
MILES

(a) Isolated, diploid populations are found in high alpine localities.

(b) Tetraploid populations are nearly always subalpine and usually confined within the limits of cultivation in Switzerland, Poland and France. Favarger and Villard (1965) state that in the Tyrol and Pyrenees, Chrysanthemum ircutianum Turcz. grows in natural habitats, where, in Switzerland, one expects to find the hexaploid chromosome race. They attribute this finding to absence of the hexaploid race in those regions.

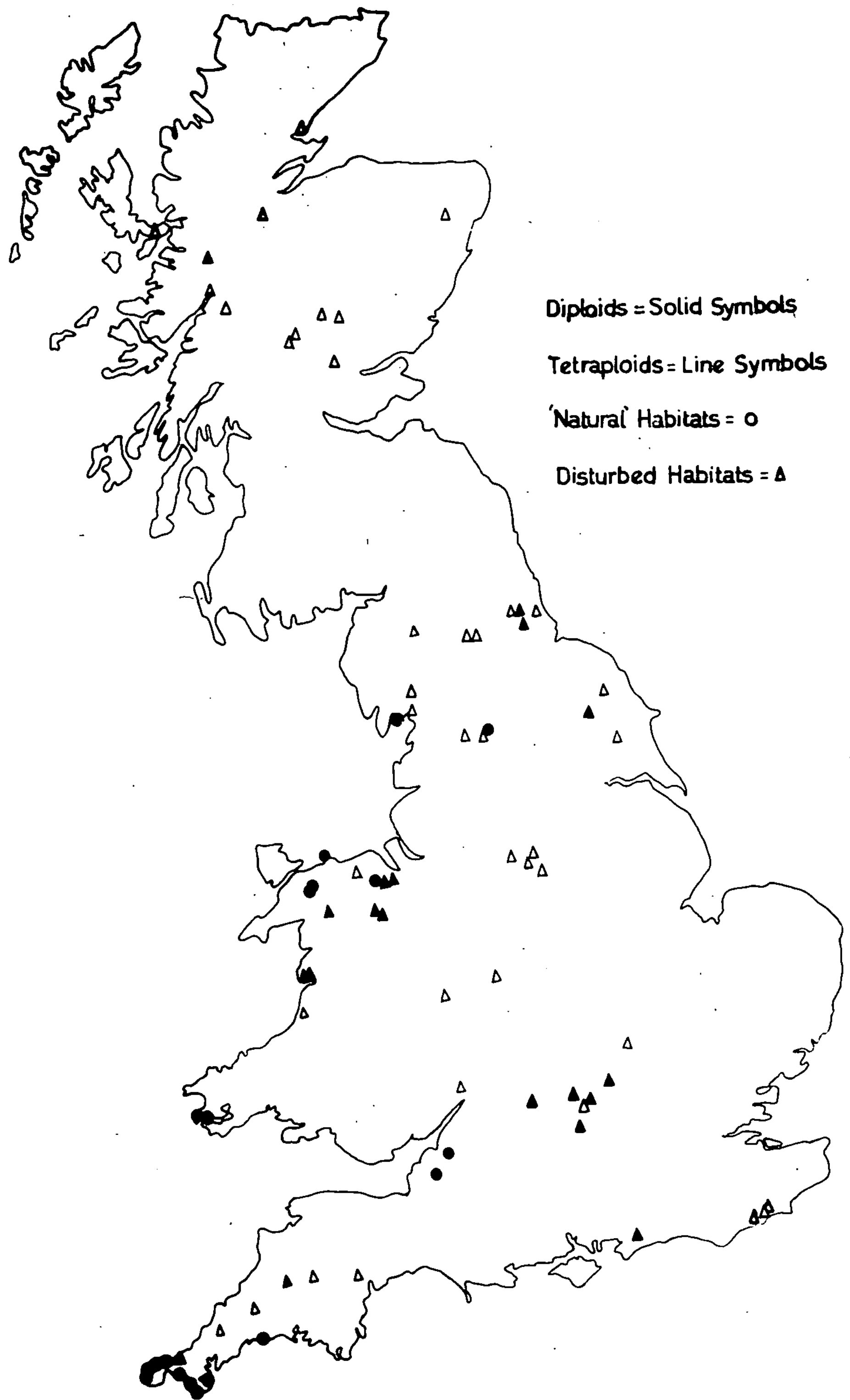
(c) Diploid populations with some morphological differences from the alpine plants are found in similar ecological conditions to the tetraploids.

(d) Hexaploids have a wide ecological tolerance, with the greater majority of plants being found above 700 metres.

My own work has shown that in the British Isles, Chrysanthemum leucanthemum L. has a distribution with certain similarities to the continental situation outlined above. Diploid and tetraploid populations are present and up to now no higher polyploid populations have been found. This is shown in fig. 38 page 58. An arbitrary distinction has been made between natural and disturbed communities, this being based upon an assessment of whether the populations sampled were found in habitats which have resulted from intervention by man.

The following points emerge:-

(a) Diploid populations are the only cytotypes found in "natural" habitats.



A CYTOTYPE DISTRIBUTION OF CHRYSANTHEMUM LEUCANTHEMUM L.

(b) Both diploid and tetraploid populations are found in disturbed habitats, and indeed some populations have proved to be a mixture of the two cytotypes. The overall correlation of diploidy with "naturality" of habitat is +.46 which is significant at the 1% level.

It might be convenient to consider in detail the habitat and community characteristics of stations sampled in Great Britain.

'Natural' Habitats

1. Sea cliff habitats Isolated diploid populations of Chrysanthemum leucanthemum L. have been found in Cornwall and North Western England and Wales, frequently growing on exposed promontories. These include The Lizard, Great Orme and Humphrey Head, figs.39 page 60. A common feature of these last named localities is the high soil pH as indicated by their species content, lists of which are given in AppendixVIII page 176. Coombe, and Frost, (1955) record a pH of 6.0 to 7.0 for the soil overlying the serpentine on the Lizard. Conversely, a low calcium status prevails. Steele (1955) has shown that magnesium replaces calcium as a principle exchange cation on serpentine soils. The Great Orme and Humphrey Head are Carboniferous Limestone headlands.

The vegetation of the Lizard localities corresponds to a Southern-oceanic Heath Type, Tansley (1939), and in this respect resembles the other cliff top localities of Cornwall and Pembrokeshire sampled. Characteristic vegetative components are Ulex gallii, Ulex europaeus, Calluna vulgaris, Erica cinerea, and at times, Erica vagans, all of which Chrysanthemum leucanthemum L. has to compete against, fig.40 page 60.

Fig. 39. Humphrey Head, Westmorland. Chrysanthemum leucanthemum, Geranium sanguineum and Armeria maritima.

Fig. 40. Lizard Cornwall. Ulex gallii mixed heath.

On the serpentine localities of the Lizard there is a vegetational transition from the front to the rear of the cliff associated with a decrease in exposure in which the dominant procumbent species of Festula ovina, stunted Calluna vulgaris, Minuartia verna, and Armeria maritima give way to Erica vagans, Ulex galli and Ulex europaeus. These communities are identical to the ones described by Coombe and Frost (1955) which they aptly name Festuca ovina-Calluna Heath or rock heath and Erica vagans - Ulex europeaus heath or mixed heath. Chrysanthemum leucanthemum is a constant component of both communities and has adopted itself in an interesting fashion to the different environments. On the rock heath, the species has adopted a dwarf habit and even when flowering, plants of only two to three inches in height are common as opposed to the normal flowering height of about 18 - 2o inches.* Another species occasionally found on the rock heath which has also adapted itself to the exposure is Sarothamums scoparium ssp. prostratus. Although of small stature, many of the Chrysanthemum leucanthemum L. plants found appeared to be fairly extensive, and, one must assume, have reproduced clonally for several years at least. Coombe and Frost (1955) point out that about one-fifth of the species composing rock heath communities are either annual or biennial. This must result in

---

* The rock heath plants were flowering at the end of April in 1966 and although the mild winter and spring of 1966 and the southern latitude may partially account for this, such precocious flowering, preceding the normal flowering time of the species by some three to four weeks in the south of England, must be regarded as exceptional.

a high turnover of individuals and although at any one time the heath presents a closed sward, many small seedlings of Chrysanthemum leucanthemum L. can be found, indicating a high rate of reproduction by sexual means. In an environment where selection pressures must be both fluctuative and extremely rigorous, such a flexibility in reproductive processes must give Chrysanthemum leucanthemum L. a good chance of survival. An interesting point of comparison is that although the 'rock heath' form of Chrysanthemum leucanthemum L. has a vastly reduced leaf area and hence reduced photosynthetic and transpiring areas, there is little or no associated reduction in the size of the flower heads or number of florets per capitulum. This is shown in fig. 41 page 63, where plants taken from the rock heath were grown in the botanic garden under controlled conditions. The results showed that the plants retained some of their characteristics, indicating that the 'rock heath' plants are probably good ecotypes in the sense of Turesson (1929). To be definite on this point one would really require a full year's growth in the Botanic garden, the growth period of the experiment mentioned being confined to one summer only, over which time there could still be some carry-over effect from the original environment.

The Chrysanthemum leucanthemum L. plants growing in the mixed heath are much taller than their rock heath counterparts. This is due to two reasons which are to some extent related.

1. The vastly reduced exposure permits taller growth.

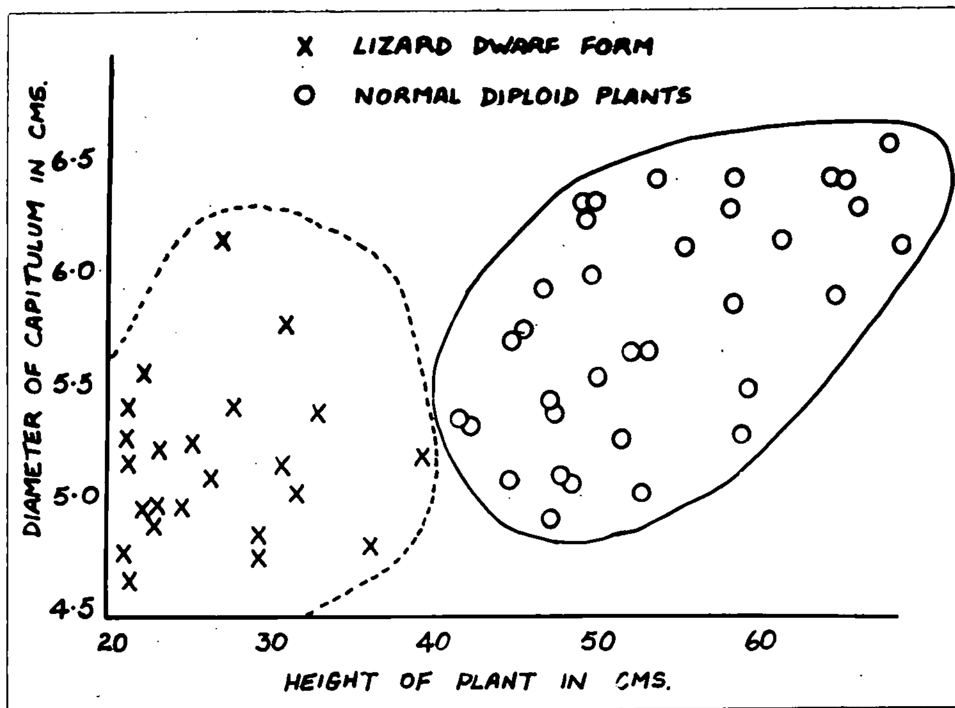


Fig. 41. Scatter diagram of height of plant against diameter of capitulum.

2. Taller growth is a necessary requisite for competition against the 'bushy' heathers and gorse which predominate in the rock heath vegetation.

In some areas there are transition zones between rock heath and mixed heath communities in which patches of Ulex europeaus and Erica vagans give way to open areas predominantly of a Festula ovina sward, containing the dwarf Chrysanthemums. Near Gunwallo Church Cove, rabbit grazing has kept open certain areas of 'mixed heath' vegetation, so that little shelter is afforded to herbaceous plants. Here also, dwarf forms of Chrysanthemum leucanthemum L. can be found growing next to taller plants, figs. 42 and 43 , page 65. The possible outbreeding of the species and the close proximity of the two types must at times result in gene flow between them; this raises the question of the stability of such populations, and whether they can be regarded as polymorphic, in the sense of Ford (1953). Old records indicate that the dwarf form of Chrysanthemum leucanthemum L. occurs at several places along the Cornish coast and hence must be regarded as widespread, and not just a pecularity of the Lizard populations sampled. Plantago coronopus var. pygmaea is similarly widespread in exposed situations and occurs frequently in Cornish cliff habitats.

The remaining cliff sites sampled had a flora which contained some of the common and a few of the rarer limestone species of Northern England and should perhaps be floristically grouped with the next type of 'natural' habitat to be examined.

Fig. 42. Dwarf forms of Chrysanthemum leucanthemum with Plantago coronopus, Sedum acre, Leontodon autumnalis.

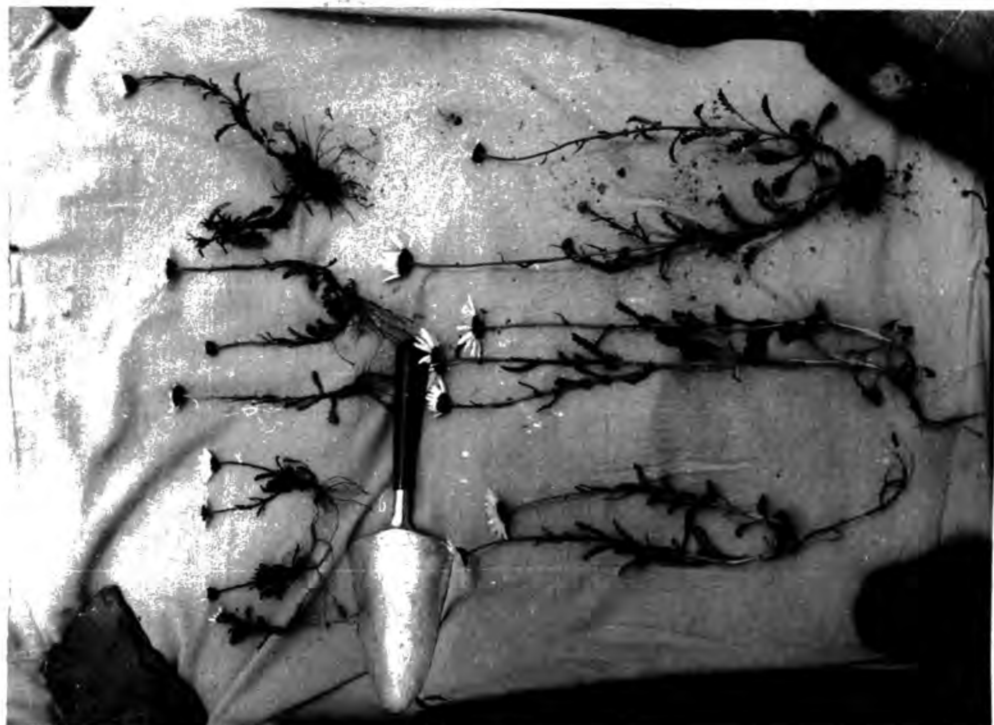


Fig. 43. A variety of growth forms of Chrysanthemum leucanthemum collected from the same population.

2. Limestone escarpment habitats Populations of Chrysanthemum leucanthemum L. have been found on rock ledges at altitudes of between 500 feet and 1,500 on the North Lancashire, Flintshire and Mendip limestone. Usually the plants grow in situations where they are not subject to grazing by sheep, and are frequently found above the tree line or on open cliff faces. On the North Lancashire limestone the tree line has been artificially depressed by man by forest clearance, probably to produce larger areas suitable for sheep grazing. The population at Malham Tarn, although of compact size, has a few plants with enormous rooting systems indicating a life span of at least several years. This limestone pasture distribution is similar to that of several species, notably Helianthemum canum (L) Baumg., Geranium sanguineum L. and Minuartia verna (L) Hiern. This latter species also occurs in one of the Lizard communities sampled. On the basis of this, one might predict that the species should occur on Upper Cronkley Fell in Teesdale, and although I have never seen the species there, old records indicate that it did grow there in the past.

3. Mountain Habitats. Several populations of diploid Chrysanthemum leucanthemum L. grow in Snowdonia at various altitudes from 1,000 feet in Cwm Idwal to 2,200 feet on the sides of Carnedd Daffydd. Floristically the communities have a strong alpine element but differ from the upland communities of the limestone escarpment. Such differences are probably due to the increased general altitude and the igneous composition of the underlying rock formation. In Appendix IX, page 177 is given a

species list of the community members found in Cwm Idwal at around 1,000 feet. Comparable populations have never been found or recorded for the Lake District, or Scottish Highlands. All accounts of the vegetation of such regions mention Chrysanthemum leucanthemum L. as being confined within the limits of cultivation. A point which may explain the presence of Chrysanthemum leucanthemum L. in the Snowdon range is the occurrence of areas of basic basalt. Many of the alpine plants growing in Cwm Idwal are recognised calcicoles and indicate a high base status. On the same grounds, one might expect the species to grow on Ben Lawers. If it does so, it is extremely rare and is probably not recorded, being usually regarded as a weed of cultivation. I have never found the species on the mountain but have collected several population samples from cultivated areas in the Lawer's region; these were all tetraploid.

4. 'Disturbed' habitats The habitats of this category include hay meadows, roadside verges, railway embankments, dry stone wall and quarry floors, etc. Both diploids and tetraploids seem to be equally successful in colonising such habitats, with a possible regional preponderance of one cytotype over another. For example, only diploid populations have been found in Flintshire, whilst around the cities of Oxford and Durham both diploid and tetraploid populations grow in equal profusion. The factors which determine the success of the species in such habitats are as follows:-

(a) Chrysanthemum leucanthemum L. is a successful coloniser of freshly exposed ground. It appears to be one of the first larger herbs to become established on new roadside embankments and between the rows of newly cut corn-fields.

(b) Most of the 'disturbed' habitats in which it is established are regularly mown throughout the growing season. The strong perennial habit of the species permits rapid vegetative regeneration and in many localities the plants will never be permitted to set seed. The coarse cutting procedures employed will rarely "mow" the species out of a community, and in fact may stimulate vegetative reproduction.

Floristically, 'disturbed' habitats can range from the characteristic flora of limestone grassland to the relatively calcifuge character of railway embankments or roadside verges in the Highlands of Scotland. There is no apparent correlation between polyploidy and type of habitat.

Present work on continental material

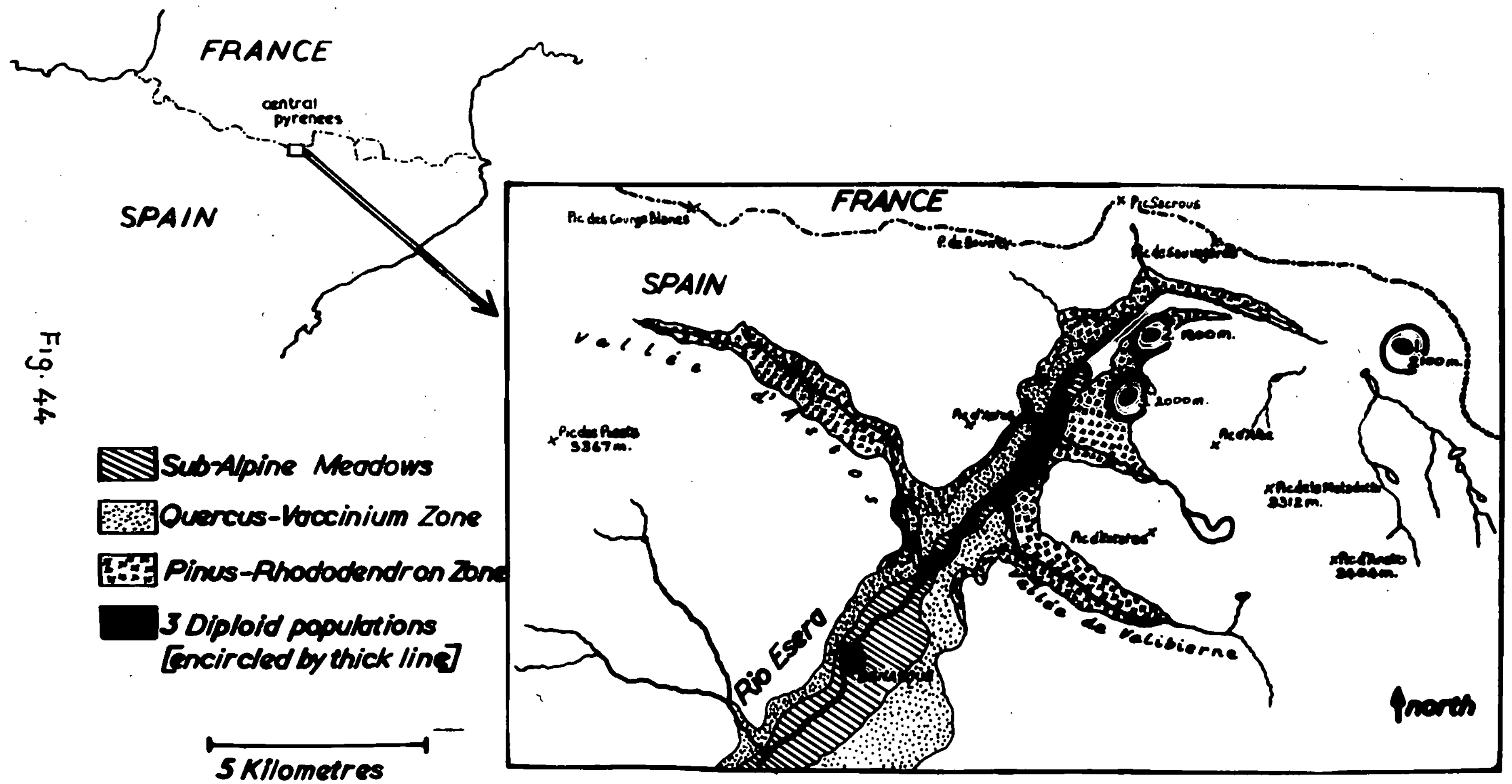
Populations from 41 localities have been sampled. Some of these have been obtained through the seed-exchange service but 30 have been collected personally or by colleagues. In the summer of 1963 I was able to go to the Central Pyrenees to investigate whether there was a similar ecological distribution of cytotypes to that described by Favarger (1959) for the Swiss Alps. Collections were made en route through France and the main centre of botanising was around the

Rio Esera, (Fig. 44, page 70) in Aragon, Spain.

The following points can be made:-

1. Chrysanthemum leucanthemum L. is first encountered above 500 metres in the hayfields of the foothills. It does not appear to be found at a lower altitude on the Spanish side of the Pyrenees. Below this altitude the character of the native vegetation changes dramatically to an arid, xerophytic type, at least in June and July, and this is associated with a sudden drop in the rainfall. From that point, the species extends to the upmost limits of cultivation at about 1,600 metres and is a most consistent member of the hayfield vegetation. Subsequent cytological analysis has shown that these populations are tetraploid, no diploids being found. At this altitude the dominant component of the natural vegetation was a Vaccinium-Quercus scrub which in places, noticeably the south-eastern side, was giving way to a pine zone. The Rio Esera ran in a south-westerly direction and the distribution of surrounding peaks was such that the north-western wall of the valley received proportionally far more hours of sunlight than did the south-eastern wall. This has resulted in a major difference in vegetation at comparable altitudes between the two sides of the valley, so that the Quercus-Vaccinium zone extended nearly 300 metres higher on the north-western side than it did on the south-eastern side. Fig. 45, page 72.

Fig. 44



2. Three isolated populations of Chrysanthemum leucanthemum were found in the positions marked on the map, fig.44 page 70. Populations 2 and 3 were found in the upper pine zone and hence must still be regarded as subalpine. They were both situated on rocky outcrops projecting out of the tree zone at 1,800 and 2,000 metres respectively, fig.46 page 72. Population 1 was found above the tree zone at about 2,100 metres in a Sesleria coerulea dominated community, on a limestone outcrop. This community was truly alpine and was closely associated with other communities containing such marker species as Chrysanthemum alpinum, Gentiana verna, Sempervivens montanum, Rhododendron ferrugineum. A species list of communities 1 and 2 is given in AppendixVIIpage 174. All three populations of Chrysanthemum leucanthemum L. were limited to some ten to thirty individuals, and proved to be diploid when samples were cytologically screened.*

One hexaploid population was found in the foothills of the Pyrenees near Jaca at a height of 700 metres. The vegetation here was distinctly xeromorphic and completely unlike any other Pyrenean habitats of Chrysanthemum leucanthemum L. seen. Lavendula latifolia was a major species of this particular community. Fig. 47 page 73.

Discussion

The ecological distribution of Chrysanthemum leucanthemum L. in

---

* A local Spanish botanist, who knew the region well, assured me that Chrysanthemum leucanthemum L. did not grow outside the limits of cultivation in that part of the Pyrenees. This indicates the rarity of the three populations sampled. Indeed, two weeks searching were required to find them.

Fig. 45. Abies alba extending down from the south-eastern side of the Vallée de Rio Esera. On the right, at a higher altitude, is a mixed Quercus-Fagus scrub.

Fig. 46. Chrysanthemum leucanthemum growing on a rocky outcrop at locality 2, Vallée de Rio Esera.

Fig. 47. A hexaploid population of Chrysanthemum leucanthemum L. sensu lato. associated with Lavandula latifolia and Ulex Europeaus at 700 metres.

Vallée de Rio Esera of the High Central Pyrenees corresponds well to the situation found by Favarger (1959) in the Swiss Alps and leaves little doubt that the Swiss situation is being repeated in that part of the Pyrenees. Skalinska et al. (1963) state that the ecological distribution of Chrysanthemum leucanthemum L. cytotypes in the Tatra also parallels those of Favarger's. One is left with a distinct impression that such an ecological orientation of cytotypes is being repeated throughout the mountain chains stretching through the Pyrenees, Jura, Alps and Tatra. As a generalised statement, this is probably true, but when considered in detail there are some rather worrying discrepancies. For example, Favarger (1959) attaches great importance to the ecological integrity of the cytotypes, but considering the published data of his own, Baksay, (1957) Skalinska (1961, 1963, 1964) Villard and Favarger (1965, 1966) and the present work, the following points arise:

A. Diploids in Switzerland cover a wide range of communities, and are found at two main altitudes:

(1) The varieties autumnale and lobatum are found normally below 600 metres growing on the edge of roads, woods and wasteland and in hayfields. These are also habitats for the tetraploid varieties pratense and praestans and Favarger and Villard (1966) note that in the Val-de-Ruz the variety lobatum is sympatric with the tetraploid.

(2) Ssp. alpicola is found at two different altitudes, fig. 2,

page 31, of Favarger (1959). About these he comments "on rencontre ces derniers, d'une part dans les pelouse alpines (entre 2000 et 2400 in d'altitude) d'autre part dans des stations sèches de l'étage montagnard .... Cela semble indiquer que dans la race diploide, il y a au moins deux écotypes, l'un de caractère alpin, l'autre plus ou moins xérophile-thermophile".

B. Skalinska (1963) points out that in the Polish Tatra the ssp. alpicola is only represented by small isolated colonies confined exclusively to the higher elevations, and there are no comparable populations to the low elevation "xérophile-thermophiles" of Favarger. The only vegetation details given refer to populations on stony slopes bearing only a scanty population at about 1,500 metres and to others in the Pinus montana zone of the Pass pod Kopa. Neither of these habitat types seems to fit the Swiss alpine situation.

C. Baksay (1957) records a diploid form commonly found at 700 metres in the hills of central Hungary. Favarger (1959) interprets these populations as being comparable to his "xérophile-thermophile" populations. He points out that Veronica prostrata also grows in two of the Swiss diploid localities and that this species has a distribution "pannonique-pontique". Unfortunately for his arguments of an alpine-central European distribution for the "xérophile-thermophile" populations of Chrysanthemum leucanthemum L. the subsp. of Veronica prostrata involved at one of the two localities is subsp. Scheereri which Brandt (1961) has shown to have a subatlantic distribution. On the other hand,

the other locality involving _Veronica prostrata_ has the subsp. _prostrata_ which according to Brandt (1961) has an alpine-Central European distribution.

D. The diploid populations of the Rio Esera in the Central Pyrenees do not seem to be identical to any of the published descriptions for other regions. If anything, they correspond to the alpine localities described briefly by Skalinska (1963), but without an accurate description of the latter it is hard to make a direct comparison.

It can be seen that the ecological distribution of diploid populations on the continent is most intricate. The apparent distributions of hexaploid populations, although perhaps not quite so confusing, also vary in different geographic regions. The reasons for this variation are easy to speculate on but less easy to substantiate. Two obvious answers might be:-

(1) Ecological differentiation of cytotypes has gone on independently in different regions in a more or less parallel fashion, or

(2) The species aggregate had an identical ecological differentiation of cytotypes over the whole geographical range and subsequent depauperisation of the populations resulted in survival of populations which had slightly different ecological preferences in different regions. Skalinska (1963) supports the last view and she states quite explicitly that "the populations of the Tatra Mountains differ in respect of their genotypic composition from those occurring in Switzerland". Certainly

Favarger's (1959) contention that "combinée avec l'écologie la cytologie permet de classer d'une manière naturelle" cannot be realised easily when the whole geographical range is considered.

The distribution of cytotypes in Britain is difficult to compare to the continental alpine distribution, since there are no directly comparable communities to the ones in which Chrysanthemum leucanthemum L. sensu lato grows in the Tatra, Alps and Pyrenees. Several of the few British habitats which are regarded as having alpine affinities also have diploid populations of Chrysanthemum leucanthemum L. whilst in most, the species is conspicuously absent. It seems more profitable to compare British populations to the continental situation in terms of populations affected and not affected by man. On the continent, diploid and hexaploid populations inhabit both disturbed and undisturbed communities whilst in Britain only diploid populations inhabit both disturbed and 'natural' communities.

A factor which seems to be common to both British and Continental diploid 'natural' habitats is the dry substratum* which is frequently limestone. Webb and Hart (1945) have suggested that preference for a calcicole habitat may be really a preference for dry soil. This might explain the absence of the species in 'natural habitats in the Lake District and Scottish Highlands.

---

* A British exception to this is the Carnedd locality in Snowdonia where the habitat was a wet rock ledge.

The south-western cliff populations are probably part of the coabtal or 'Atlantic' distribution of diploids referred to by Böcher and Larsen (1957). It is possible that an 'Atlantic' distribution has no biological meaning apart from the fact that many cliff communities are natural and in conjunction with many other undisturbed communities act as sites for the diploid cytotype.

Regarding the distribution of the tetraploid cytotype, there is universal agreement. With the exceptions pointed out on page 57, all workers have found it consistently associated with disturbance of natural vegetation. In Britain several localities have been found in which diploids and tetraploids are sympatric. This is similar to the situation described in the Val-de-Ruz by Villard and Favarger (1966) and is unlike that described by Favarger (1959) in the Vallée de La Brevine where the two races cohabit but are ecologically differentiated, and where ecological differences can be detected. In Durham City a diploid and tetraploid plant have been found within 5 yards of each other.

Conclusions.

There is an ecological differentiation of chromosome races of the species aggregate Chrysanthemum leucanthemum L. sensu lato which is associated with the disturbance of natural vegetation. In natural localities in the British Isles only diploid populations are found. The situation concerning the ecology of the lowland diploids and tetra-

ploids is not so satisfactory in that there is a lack of criteria for distinguishing habitats of the two cytotypes. This same pattern has been described in Switzerland by Villard and Favarger (1966).

S E C T I O N   III

AN ANALYSIS OF BREEDING BEHAVIOUR

IN

CHRYSANTHEMUM LEUCANTHEMUM L.

SENSU LATO

AN ANALYSIS OF BREEDING BEHAVIOUR IN CHRYSANTHEMUM LEUCANTHEMUM SENSU LATO

A series of hybridisation experiments was carried out to investigate:-

1. the nature of the breeding system in Chrysanthemum leucanthemum L. under experimental conditions.
2. the genetical similarities of diploids and tetraploids by establishing triploid hybrids and examining their meiosis for chromosome pairing behaviour (the results of this particular experiment have already been reported in the section on Cytology).
3. the genetical similarity within the diploid and tetraploid levels.

The small size of the hermaphrodite disc florets made it impossible to emasculate them and consequently in the first instance only the outer female ray florets were used. This was achieved by removal of all the disc florets before maturation of any of the anthers and then pollination of the stigmas on the female florets after their emergence. The flower heads were enclosed in cellophane bags to prevent spurious pollinations. This particular technique proved disastrous for two reasons:- (a) the conditions inside the bags i.e. a high humidity and open tissue, were ideal for invasion by Botrytis.
(b) the damage sustained by the ray florets during removal of the disc florets may have prevented the seed from setting.
Accordingly a repeat hybridisation program was carried out, but this time employing the whole capitulum and dispensing with pollinating

bags by use of an insect-proof greenhouse. In all, over 500 hybridisations were performed. At least one capitulum per plant was left alone to assess the rate of self fertilisation.

The conclusions of the hybridisation program are as follows:

A) Self pollinations resulted in a low average seed set (1-8-41). Excluded from this data are two continental plants which set 100% of their seeds. These plants may well have been apomictic or inbreeders. There were no differences between polyploid levels in the amount of self fertilisation.

B) There were no significant differences in seed set between reciprocal crosses. Consequently the following results include the summated data for reciprocal crosses. To minimise the affect of environment only data has been included for those crosses in which there was one or more seeds set.

C) 2n x 2n crosses yielded an average seed set of 55 seeds per capitulum (2-55-115).

D) 4n x 4n crosses yielded an average seed set of 51 seeds per capitulum (2-51-168).

E) 4n x 2n crosses resulted in an average seed set of 23 seeds per capitulum (1-23-114).

F) Auto. 4n x 4n crosses resulted in an average seed set of 45 seeds per capitulum (11-45-108).

These data have been statistically compared and this is shown in Fig.48 page 82.

COMPARISON OF SEED SET IN DIFFERENT CROSSES

	4N x 4N	4N x 2N	2N x 2N	SELF	AUTO x 4N
4N x 4N	2-)61(-168	1-)23(-114 P=0·01	2-)56(-115 N.S.	1-)8(-41 P=0·05	11-)46(-108 N.S.
4N x 2N			P=0·01	P=0·01	P=0·05
2N x 2N				P=0·01	N.S.
SELF					P=0·05
AUTO x 4N					

N.S. = NOT SIGNIFICANT

AUTO = SYNTHESISED TETRAPLOID

Fig. 48

The following table gives the percentage of crosses which gave no seed set.

Percentage of crosses which gave no seed set	
2n x 2n	= 12%
4n x 4n	= 18%
4n x 2n	= 15%
selfed	= 55%
Auto 4n x 4n	= 8%

Germination of the seeds produced by the previous experiments led to the following conclusions:-

A) There was a high germination-rate of seed resulting from the 2n x 2n and 4n x 4n crosses. This varied from between 50% to 90%.

B) There was a low germination rate of seed resulting from triploid crosses. This varied from between 0 to 20%. Subsequent cytological analysis of the seedlings showed that most of the seed formation had resulted from selfing and was, therefore, not of hybrid origin. In fact only 15 plants out of a total of 300 proved to be triploid. An interesting point arising from these data is that the rate of self pollination is comparable in attempted 2n x 4n crosses to the average rate of selfing for 2n or 4n plants left to their own devices. This indicates that cross pollination between polyploid levels has not broken down the self incompatibility mechanisms of the female parents. Examination of the inviable seed showed that there was no endosperm

formation, which suggests that the seed was of triploid hybrid origin.

C) The 15 triploid hybrids were produced in three different hybridisations all involving British diploids and Continental tetraploids. In two of the hybridisations, the tetraploid was the female parent. In the remaining hybridisation, production of triploid seed by self fertilisation with unreduced pollen can be eliminated, since the progeny resulting from germinating the seed completely resembled the tetraploid parent. In this instance the tetraploid parent was morphologically distinct from the diploid parent.

Discussion

In the greenhouse, Chrysanthemum leucanthemum L. appears to be an outbreeder with a facility for a low level of inbreeding. It is probable that a similar breeding system exists in nature, although this still remains to be proven.

Stebbins (1957) has remarked that nearly all the examples of complete or nearly complete autopolyploidy are in species which are extensively outcrossed in nature. Lewis and John (1963) have explained this by suggesting that since auto-polyploidy can arise either by production of unreduced gametes or by somatic doubling and that since unreduced gametes are likely to be at a disadvantage in a self compatible diploid, then the most probable origin of autopolyploidy is by somatic doubling of an outbreeding diploid. This explanation has one loop hole since presumably, autopolyploidy could also arise from somatic doubling of self compatible diploids.

Tetraploid Chrysanthemum leucanthemum L. has many similarities to known autotetraploid cytological behaviour. Amongst its own cytological peculiarities is the phenomenon of localisation and reduction in number of chiasmata to one per chromosome arm in a distal position. This results in long, undisturbed linkage groups situated on each side of the centromere. In an inbreeding organism it seems likely that this particular cytological situation would be disadvantageous, since homozygosity would be quickly established. Hewitt and John (1964) has demonstrated the converse situation in isolated populations of grasshoppers, which are so small as to be considered inbred. Here, increased rates of inbreeding are compensated by increases in the number of chiasmata.

Possession of a very low level of inbreeding confers the potential for sexual fitness in isolated individuals of Chrysanthemum leucanthemum L. It is likely that the experimental plants which were fully self-compatible were homozygous for a recessive gene promoting inbreeding. Similar conditions exist in Tripleurospermum maritimum, Q. Kay, (personal communication) and in Dactylis glomerata, M. Borril (personal communication).

Conclusions

Under experimental conditions Chrysanthemum leucanthemum L. appears to be mainly outbreeding with a low level of inbreeding. This

situation may be related to a meiotic mechanism involving localisation of chiasmata and reduction of genetical recombination. It is suggested that such a mechanism could only persist in an outbreeding organism.

SECTION IV

VARIATION OF PHENOLIC PIGMENTS IN

CHRYSANTHEMUM LEUCANTHEMUM L.

SENSU LATO

VARIATION OF PHENOLIC PIGMENTS IN CHRYSANTHEMUM

LEUCANTHEMUM L.
SENSU LATO

The works of Bate-Smith (1959, 1961, 1962) and Alston and Turner (1959, 1963) amongst others, have demonstrated the value of plant chemical components as taxonomic characters. The above mentioned authors have mainly concerned themselves with phenolic components extracted from leaves. Detection and identification of the components was carried out using paper chromatographic techniques.

It was decided to investigate the phenolic components in basal leaves of Chrysanthemum leucanthemum L. The method of extraction and paper chromatographic techniques recommended by Bate-Smith (1962) were employed and proved to be somewhat unsatisfactory owing to trailing effects of components. Accordingly, a thin-layer chromatographic technique was successfully substituted. The details of this are given in Appendix X page 178.

Seventy-eight plants from different sources, including triploid hybrids and their parents and synthesised autotetraploids, were analysed. The average positions and total number of spots found are given in Fig. 49 page 88 . Fig. 50 page 89 , displays some of the results.

The following conclusions can be drawn from the results:-

A) Component 9 was generally present in diploid plants and absent in tetraploid plants. In those tetraploid plants in which component 9 was found, it appeared to be present in lower concentrations as

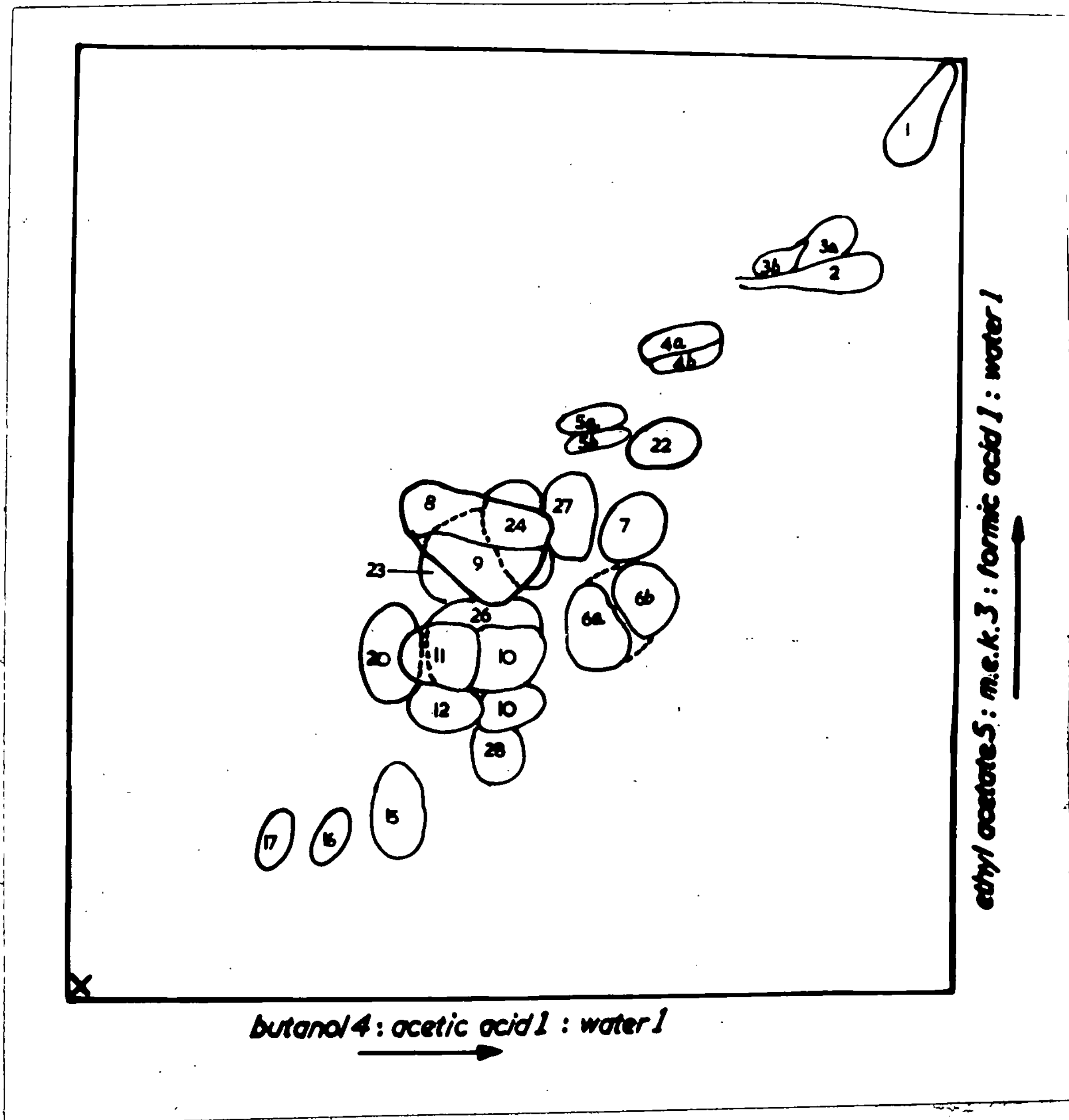
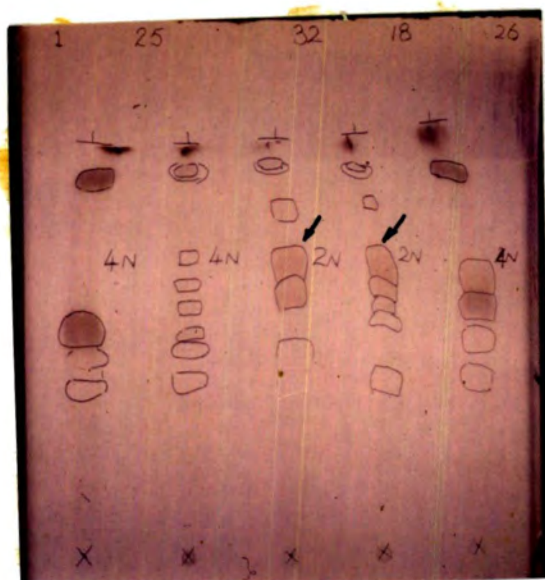


Fig. 49.

Chromatogram spots

CATEGORIES OF PLANTS	PLANT CODENUMBERS	ORIGIN OF PLANTS	2	3a	3b	4a	4b	5	6a	6b	7	8	9	10	11	12	13	15a	15b	16	17	20	21	23	24	25	26	27	28
HYBRIDS and PARENTS	862/1	Udine 4x	X	X	X	X		X	X	X	X	X		X		X		X		X	X								X
	862/1→462/1 ①	hybrid 3x	X	X					X			X		X	X							X							
	862/1→462/1 ⑥	hybrid "	X	X				X	X			X		X	X							X							
	862/1→462/1 ②	hybrid "	X	X		X			X	X	X	X		X	X							X							
	462/1	Festiniog 2x	X	X		X			X			X	X			X	X	X		X	X								
	1561/10→3062/3	hybrid 3x	X	X	X	X	X		X	X		X		X		X				X									
	3062/3	Prague 4x	X	X		X			X			X		X		X				X									
	461/5→2662/11 ①	hybrid? 4x		X	X	X			X							X		X		X	X								
	2662/11	Prague 4x		X	X	X			X	X		X				X		X		X	X								
	462/1→2662/11 ⑥	hybrid 3x	X	X		X			X	X		X		X		X													
	462/1→2662/11 ④	" "	X	X		X			X			X		X		X													
	462/1→2662/11 ③	" "	X	X	X	X	X	X	X	X		X		X		X				X	X								
	462/1→2662/11 ①	" "	X	X		X		X	X			X		X		X				X	X								
DIPLOIDS	1562/3	Stavropol	X	X		X		X	X			X	X	X		X	X	X		X	X	X							
	964/1	St. Ives	X	X		X			X	X	X	X	X	X	X	X	X	X		X									
	264/1	Kynance Cove		X		X			X	X		X	X			X		X		X									
	2562/3	Little Haven		X	X	X			X			X	X			X	X	X		X	X								
	2462/1	Carnedd Dafydd	X	X	X	X		X	X		X	X	X	X		X		X		X	X								
	362/9	Loggerheads	X	X	X	X			X			X	X	X	X	X	X			X									
	362/2	Loggerheads	X	X	X	X			X			X	X	X		X	X			X	X								
	462/3	Mold	X	X	X	X			X			X	X	X		X	X	X		X	X								
INDUCED TETRAPLOIDS	A2/3	colchicined	X	X		X		X	X			X	X	X		X		X		X									
	A2/9	" "	X			X		X	X			X	X			X				X	X								
	A2/4	" "	X	X		X		X	X	X		X				X	X	X	X	X	X	X							
	A2/6	" "	X	X		X			X			X	X	X		X	X	X		X	X	X							
	A2/7	" "	X	X		X		X				X	X			X	X			X									
	462/5→A2/3 ⑨	hybrid?	X	X		X	X	X	X	X	X	X		X		X		X		X	X	X							
TETRAPLOIDS	1063/6	Benasque	X	X					X	X	X			X		X	X			X	X								
	1161/9	Edale	X	X	X	X		X	X			X						X	X	X	X	X					X		
	4162/2	Malham Moor	X	X		X			X			X		X		X	X	X		X	X								
	663/1	Pickering		X	X	X			X			X	X			X		X		X		X		X					
	662/3	Warwick		X	X				X		X	X		X		X		X											
	961/1	Matlock		X	X			X	X		X	X		X		X		X											
	2862/4	Prague	X	X	X			X	X			X		X		X	X	X							X				
	3061/1	Zagreb	X	X					X		X			X		X													
	2762/2	Ottawa	X	X		X		X	X			X		X		X													
	1162/2	Cracow	X	X		X			X		X	X		X		X		X		X	X				X			X	
	3461/1	Neuchatel	X	X					X		X	X		X				X		X	X	X							X
	3262/6	Strasbourg	X	X		X			X		X	X	X	X		X		X		X	X	X							X
PYRENEAN DIPLOIDS	1263/10	Benasque		X	X				X													X		X	X				
	1163/1	Benasque		X	X				X													X		X	X	X			
OTHER SPECIES	Chrysanthemum rotundifolia	Czechoslovakia	X	X	X						X	X		X		X						X						X	
	Chrysanthemum maximum	Garden variety		X	X									X		X		X		X	X	X		X	X		X		
	Chrysanthemum maximum	Garden variety		X										X		X		X		X	X	X		X	X		X		

Fig. 50



Single dimensional chromatogram showing extra brown pigment in diploids. →

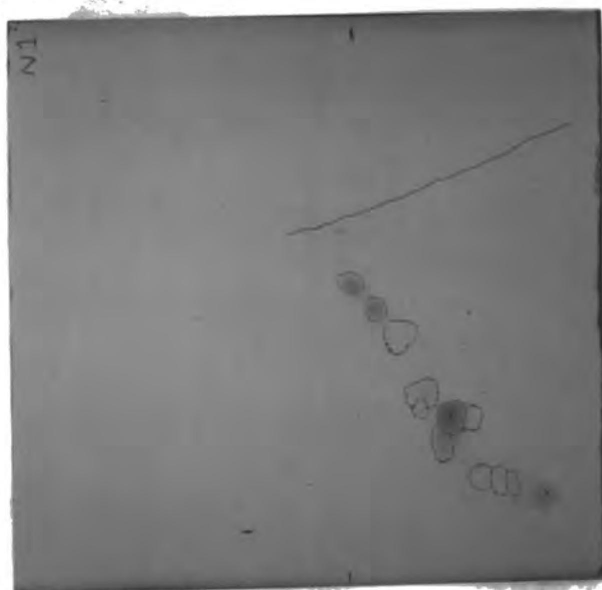
Two dimensional chromatogram of British diploid. The large spot represents compounds 8 and 9 of Fig. 49. →

Two dimensional chromatogram of British tetraploid. There is no large brown spot.

Two dimensional chromatogram of triploid hybrid. There is a small brown spot. →

Fig. 51A.

Two dimensional chromatogram of synthesised tetraploid. Note the possession of a large diploid-type brown spot. →



Two dimensional chromatogram of a Continental tetraploid. There is no large red-brown spot corresponding to spot 8 and 9.

Fig. 51 b.

demonstrated by size and intensity of spot. Solutions of component 9, obtained by eluting out the component from the Silica Gel with Ethanol, changed colour with changes in pH. The solutions turned blue with increasing pH and to yellow with decreasing pH. Such features are typical of leuco-anthocyanins. Rf. values indicated that the compound could have been leuco-cyanidin. Component 8 was located next to component 9 on the chromatograms and unfortunately had similar chemical and physical properties. It had a slightly higher Rf. value and could have been leuco-pelargonidin.

B) No triploid hybrids contained component 9 and in general had a smaller total number of components than the parent plants. They appeared to more closely resemble the tetraploid parental pattern, a fact which was corroborated from other sources, page 84.

C) Synthesised tetraploids appeared to resemble the parental diploid pattern. However, they had component 8 in smaller concentrations than the diploid parents.

D) Pyrenean diploids had a completely different pattern to all other diploids and tetraploids. Their total number of detectable components was much lower than in any other plants examined.

E) Specimens of <u>Chrysanthemum maximum</u> Ram. had certain similarities to the Pyrenean diploids, in particular with reference to components 20, 23 and 24.

F) <u>Chrysanthemum rotundifolium</u> did not have any different components to some of the tetraploid specimens examined.

Discussion

It seems that leaf phenolic compounds give a partial discrimination between polyploid levels in *Chrysanthemum leucanthemum L.* If, after further analyses in depth have been carried out, the pattern of variation of the phenolic components can still be shown to be of taxonomic value, then there are no *a priori* reasons why such features cannot be used in conjunction with the more traditional morphological attributes.

In the present work it has not proved convenient to incorporate phytochemical characters into the multivariate analysis (described in section 5), since collection of data for the latter work would have involved chromatographic analysis of all the plants used.

NOTE: The problem of getting sufficient material to measure all the characters likely to be used in a multivariate analysis increases proportionally to the number of characters employed. For example, the pressing of plants at a stage of development suitable for measuring leaf characters meant that mature seed characters could not be used. Similarly, in order to retain sufficient replicates of basal leaf parameters, insufficient leaves were available for chromatography.

SECTION V

MORPHOLOGICAL VARIATION IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

MORPHOLOGICAL VARIATION IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

Introduction

Any impartial survey of the taxonomic literature on this species aggregate reveals the following:-

a) There is widespread confusion resulting from regional authors having created superfluous synonyms and giving inadequate plant descriptions.

b) Too much emphasis has been placed upon apparently single characters. This is in itself a doubtful taxonomic pursuit but becomes even more so when the characters used appear to vary in an uncorrelated fashion. An example of this is quoted by both Böcher and Larsen (1957) and Favarger (1959) who point out that the presence or absence of a pappus on the peripheral achenes can vary within the same plant or even same capitulum and that in previous comprehensive surveys of the genus, viz., Briquet et Cavillier (1916) such a ludicrous distinction warranted at the least, subspecific status.

c) The confusion of previous taxonomic treatments of the genus follows a pattern peculiar to widespread critical taxa in which small morphological differences are caused either by slight genetical variants or phenotypic plasticity or both. Either root cause often results in morphological diversity of a nature most difficult to dichotomise and describe, both most necessary properties of a successful taxonomical treatment.

The cytological researches of Dowrick (1952), Böcher and Larsen (1957),

Favarger (1959), Baksay (1957), Mulligan (1959) and the present work have shown that there is cytological variation within the species aggregate and indicate that this might be used for a basis from which associated character variation could be studied. The rationale for this can be stated briefly:- with few exceptions, see page 52, differences in polyploidy result in different breeding groups and hence give the opportunity but not certainty, for differential adaptation and morphological modification to occur between polyploid levels. Should such differences prove consistent enough, then taxonomical recognition can be given. Böcher and Larsen (1957) chose to give specific recognition to the diploid and tetraploid levels, by aparting them to Chrysanthemum Leucanthemum L. and Chrysanthemum ircutianum Turcz. respectively. This course was followed by Baksay (1957) and Favarger (1959) but ignored by Mulligan (1959) who, whilst conceding that there were certain similarities between the two varieties, var. sub-pinnatifidum Lecoq. and Lamotte and laciniatum (in Grey's Manual of Botany) and the morphology of the two cytotypes in North America, remained uncommited on whether the cytotypes should receive specific recognition. In the Table J on page 94 is a list of characters considered by previous authors to characterise the two taxa in question. Because of the difficulties of transposing the descriptions used into a standardised terminology without adding to the ambiguity already present, the original terminology has been put into the Table, leaving the readers to interprete as they wish. Where it is felt there is a fair correspondence

TABLE I

A check list of characters used by authors to distinguish *Chrysanthemum leucanthemum* L. from *Chrysanthemum ircutianum* Turcz.

Characters for *Chrysanthemum leucanthemum* L. given in capitals and for *Chrysanthemum ircutianum* in small script

	De CANDOLLE	BAKSAY	FAVARGER	BÖCHER & LARSEN	FLORA ROSSICA	MANUAL OF BOTANY[3]
Branching habit	sparsely branched	sometimes branching	Nearly always branched	frequently branched	sometimes sparsely branched	No information
	BRANCHED	NO INFORMATION	BRANCHED AND UNBRANCHED FORMS	UNBRANCHED OR SPARSELY BRANCHED	NORMALLY UNBRANCHED	-
Shape of cauline leaves	oblong, obtuse	oblanceolate	No information	oblong-lanceolate to linear-lanceolate	oblong	oblong or oblanceolate
	NO INFORMATION	OBLANCEOLATE-LANCEOLATE	NEARLY ALWAYS NARROW	LINEAR-LANCEOLATE	NO INFORMATION	-
Condition of cauline leaf margins	dentate	serrate or bluntly indentate	crenate-dentate but not normally pinnatifid	regularly and closely toothed	serrated	No information
	SERRATE	NO INFORMATION	DEEPLY LACINATE TO PINNATIFID	NOT VERY REGULARLY TOOTHED	SERRATED	-
Condition of base of cauline leaves	semi-amplexicaul	broad base and sessile	enlarged base with two auricles	not or slightly pinnatifid	subincised-serrated	conspicuously subpinnatifid
	SEMI-AMPLEXICAUL, INCISED, SERRATED BASE	LOBED, DEEPLY AURICULATE	ENLARGED BASE, TOOTHED TO LOBED	PINNATIFID BASES	PROFOUNDLY PINNATIFID	-
Shape of basal leaves	No information	No information	No information	spathulate to spathulate lanceolate	obovate-spathulate	no information
	OBOVATE	NO INFORMATION	NO INFORMATION	+ or - SPATHULATE	OBOVATE-SPATHULATE	-
Condition of margin of basal leaves	No information	No information	No information	regularly toothed and not lobed	No information	pinnatifid-subpinnatifid or coarsely and irregularly toothed
	DENTATE	CRENATE-CRENATELY INCISED	NO INFORMATION	IRREGULAR ALTERNATING TEETH OR LOBES	INCISIVELY SERRATED	-
Condition of pappus	Diverse pappi	With pappus	In general without pappus	Frequently with more or less distinct pappus	Toothed medial to dorsal pappi	No information
	NO INFORMATION	UNIFORMLY FREE OF PAPPUS	IN GENERAL WITHOUT PAPPUS - BUT SOMETIMES RUDIMENTARY	USUALLY WITHOUT PAPPUS - IF SO THEN RUDIMENTARY	NO PAPPUS	-
Height of plant	No information	No information	No information	No information	No information	No information
	NO INFORMATION	SMALL STATURE	SMALL STATURE	NO INFORMATION	NO INFORMATION	-
Size of pollen	No information	No information	+ or - 33 u	30 -39 u	No information	No information
	NO INFORMATION	NO INFORMATION	+ or - 30 u	27 - 30 u	NO INFORMATION	-
Colour of involucral bracts	-	No information	No information	No information	Green-white margin and dark brown surround	No information
	-	NO INFORMATION	NO INFORMATION	NO INFORMATION	RED BROWN MARGIN	-
Habitat	No information	Meadow plant of the plains	Mountain and sub-alpine meadows	No precise information - variable	No information	Fields, meadows, roadsides
	NO INFORMATION	HAY MEADOWS, MOUNTAIN MEADOWS, SUNNY GRASSLANDS	ALPINE GRASSLANDS[1] LOWLAND FIELDS[2]	FREQUENTLY IN NATURAL PLANT COMMUNITIES	NO INFORMATION	-
Size of the flower	Larger ligules and more in number than in *C. leucanthemum*	Large infloresence	Flower heads of medium size	5 - 7.5 cms.	No information	Flower heads small
	NO INFORMATION	SMALL INFLORESCENCE	FLOWER HEADS SMALL	4 - 7 cms.	NO INFORMATION	-

1 regarded by Favarger (1959) as two separate subspecies and
2. by Favarger and Villard (1966) as separate varieties

3 var. pinnatifidum from Grey's Manual of Botany has been regarded by Mulligan (1959) and Cooper & Mahoney (1935) as being tetraploid and must be compared as such to *Chrysanthemum ircutianum* Turcz.

in meaning along rows, this has been indicated by different types of cross hatching and dissimilarity by dots. It is obvious from the table that there exist opposing discriptions and indeed the var. pinnatifidum (extreme right hand column) might be substituted with perfect agreement into the row for Chrysanthemum leucanthemum L. As far as can be assessed this split has been made within the confines of the subspecies triviale Gaudin as described by Briquet and Cavillier (1916). The latter note that Chrysanthemum ircutianum Turcz has some 'affinities' to Chrysanthemum leucanthemum L. but do not include the species in their review of the genus in the Maritime Alpes nor in their list of synonyms.

A rather surprising feature of the works by Böcher and Larsen (1957) and Favarger (1959) is the absence of any form of key for identifying the species. This ommission is probably indicative of the variable nature of the characters used in their descriptions.

Present contribution

Using the characters outlined above a survey was made of British Herbarium material*. Some 500 sheets were examined and the following conclusions were drawn:-

1. Although a proportion of the sheets could be selected which more

---

* At this point in the investigation, it was already appreciated that two chromosome races existed in the British Isles and the Herbarium sheet investigation was primarily concerned with seeing whether two morphological species could be detected amongst the sheets of Chrysanthemum leucanthemum L.

or less filled the specific descriptions of both Böcher and Larsen (1957) and Favarger (1959) a large number of specimens presented characters with intermediacy of variation or variation of a type which was incompatible with the spectrum existing between the descriptions. A perturbing feature of this analysis was the apparently uncorrelated variation of some characters. It became obvious that it was necessary to determine whether this variation might be attributed to an environmental component or to genetical segregation and a randomised growth experiment involving cytologically determined plants from a variety of populations was established, Appendix XI, page 180.

2. Although the artificial key to the varieties of the subspecies <u>triviale</u> Gaudin, in Briquet et Cavillier (1916) did not work, the general descriptions, by and large, fitted most of the variants seen both on herbarium sheets and in the field. In this context it is of interest that Villard and Favarger (1966) make use of the varieties of Briquet and Cavillier when referring to different types of populations growing in Switzerland.

<u>An analysis of character variation of cytologically identified individuals</u>

The following examples of character variation were taken from plants grown in pots in the greenhouse or in outdoor flower beds for one year, and are not the same as those taken from the randomised growth experiments, and used in the multivariate analysis, page 106. Although the specimens used were not directly comparable in their growth histories in the botanic garden, if any differentiae did exist

between polyploid levels, they should be apparent even under such conditions.

Basal leaf morphology

The parameters shown in Fig. 52, page 98 were plotted for 58 plants on a scatter diagram, Fig. 54 page 99. It is apparent that these characters are giving a partial separation of cytotypes in an overlapping distribution. Applying a form of Anderson's hybrid index to these data produces the distribution shown in Fig. 53 page 98. The separation given by the latter technique on the characters used is inferior to that shown in the scatter diagram where the characters used on the principal axies, and hence weighted, have been selected for their 'constancy'. Considerations of the rationale for weighting characters are given on page 117. The statistic for the separation shown in the Hybrid Index gives a significant difference at the 1% level. If the characters had been standardised by the procedure of transforming the standard deviation for each character to unity and allotting to each character sample a value differing from the standard deviation according to the formula $\frac{\text{S.D. - character value}}{\text{mean for all values}}$, then this would have given a better split for the Hybrid Index than that shown in Fig. 53 page 98. For this analysis the more normal procedure of allotting a zero value for one character extreme i.e. diploid and the value 6 for the other extreme i.e. tetraploid was adopted. A typical range of basal leaf morphologies is shown in Fig. 56 page 101A the salient feature being the complete continuum of variation

Fig. 52.

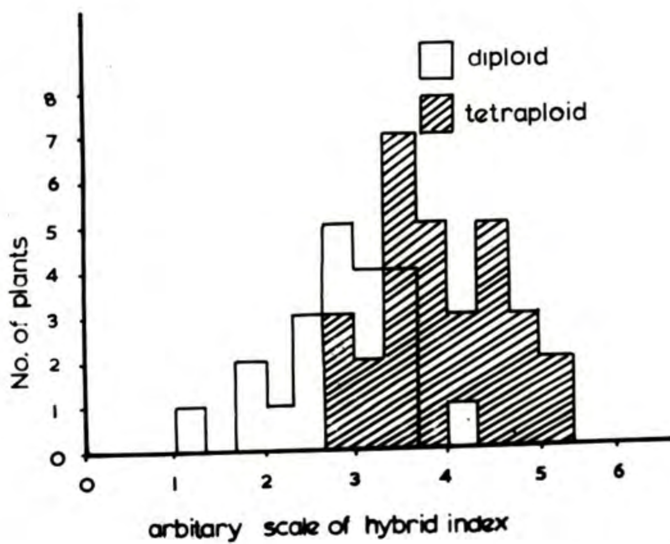


Fig. 53.

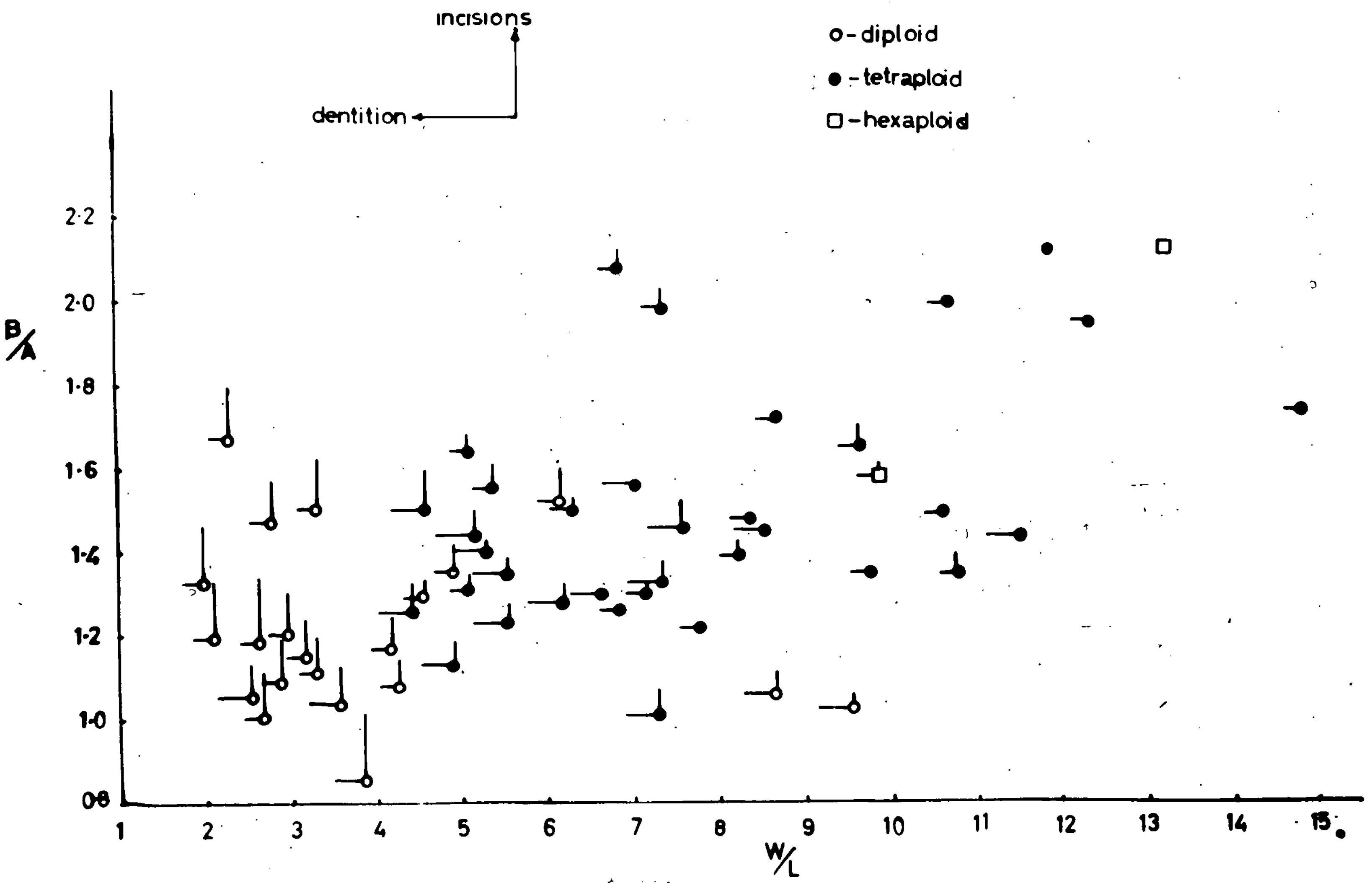


Fig. 54.

from one end to the other.

Pollen Grain Diameter

The only character for which previous workers have published any data has been pollen grain diameter. Böcher and Larsen (1957) and Favarger (1959) consider that the distribution is overlapping. Mulligan (1959) came to the conclusion that there was a complete dichotomy of pollen grain size in Canadian material between the two cytotypes. In Fig. 55A page 101 I have plotted the pollen grain diameter of 50 plants, each plant having 100 grains measured. The method used for measurement is outlined in Appendix III. The distribution is fairly similar to that shown for basal leaves in Fig. 53 page 98. The essential point about this particular diagram is that each grain measured has been included and the distributions are not made up from mean grain diameters.

Examination of mid-stem leaves showed the following:-

a) there was a tendency for the leaf incisions to be more irregular in depth and spacing in diploids than in tetraploids. Fig. 54 page 99. An exception to this were the diploid plants corresponding to the variety alpicola (Koch) Gremli, a description of which is on page. 126 . This feature of dentition and incision irregularity could also be demonstrated on basal leaves and although the basal and stem leaf conditions of such characters were obviously positively correlated there were certain exceptions, viz. variety alpicola, which produced problems of

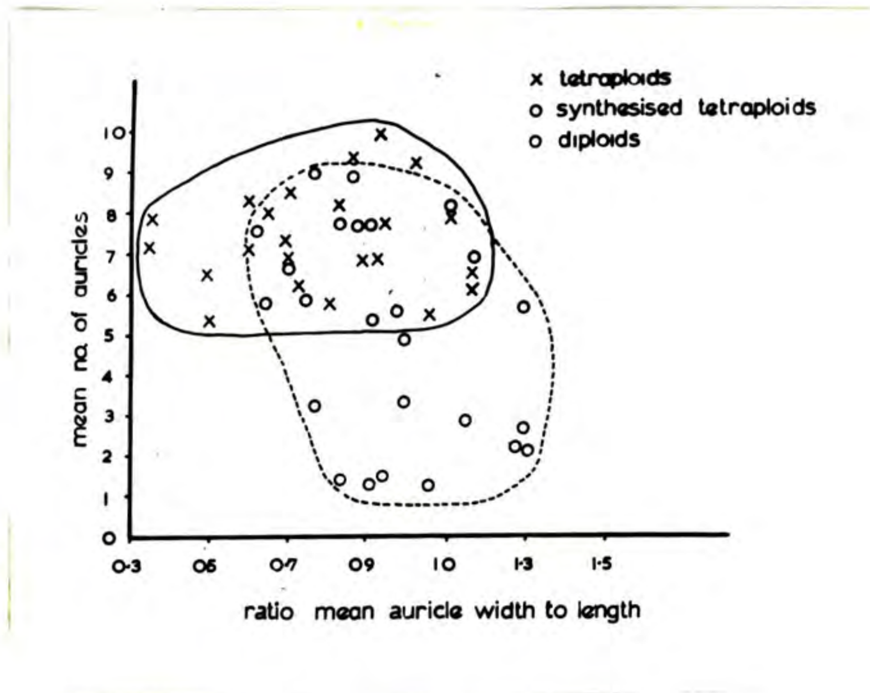


Fig. 55.

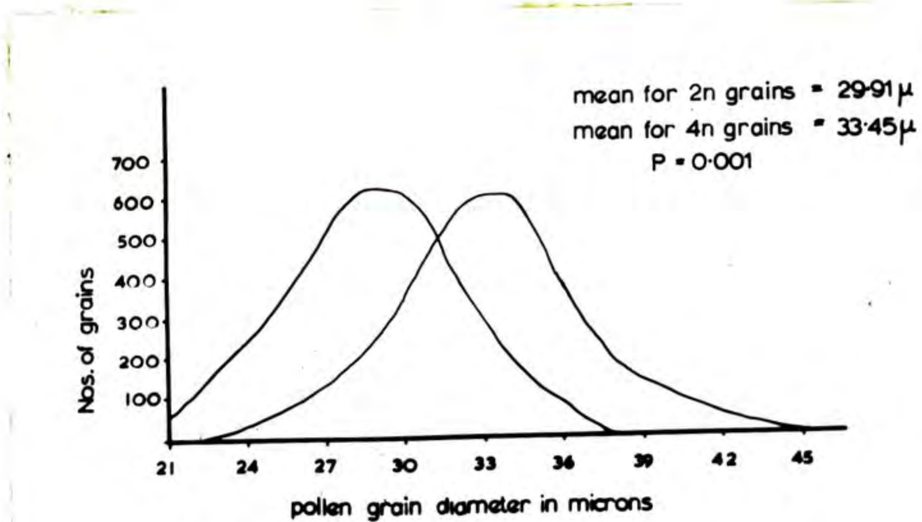
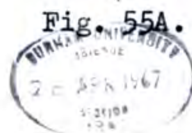


Fig. 55A.


DURHAM UNIVERSITY
SCIENCE
2 APR 1967

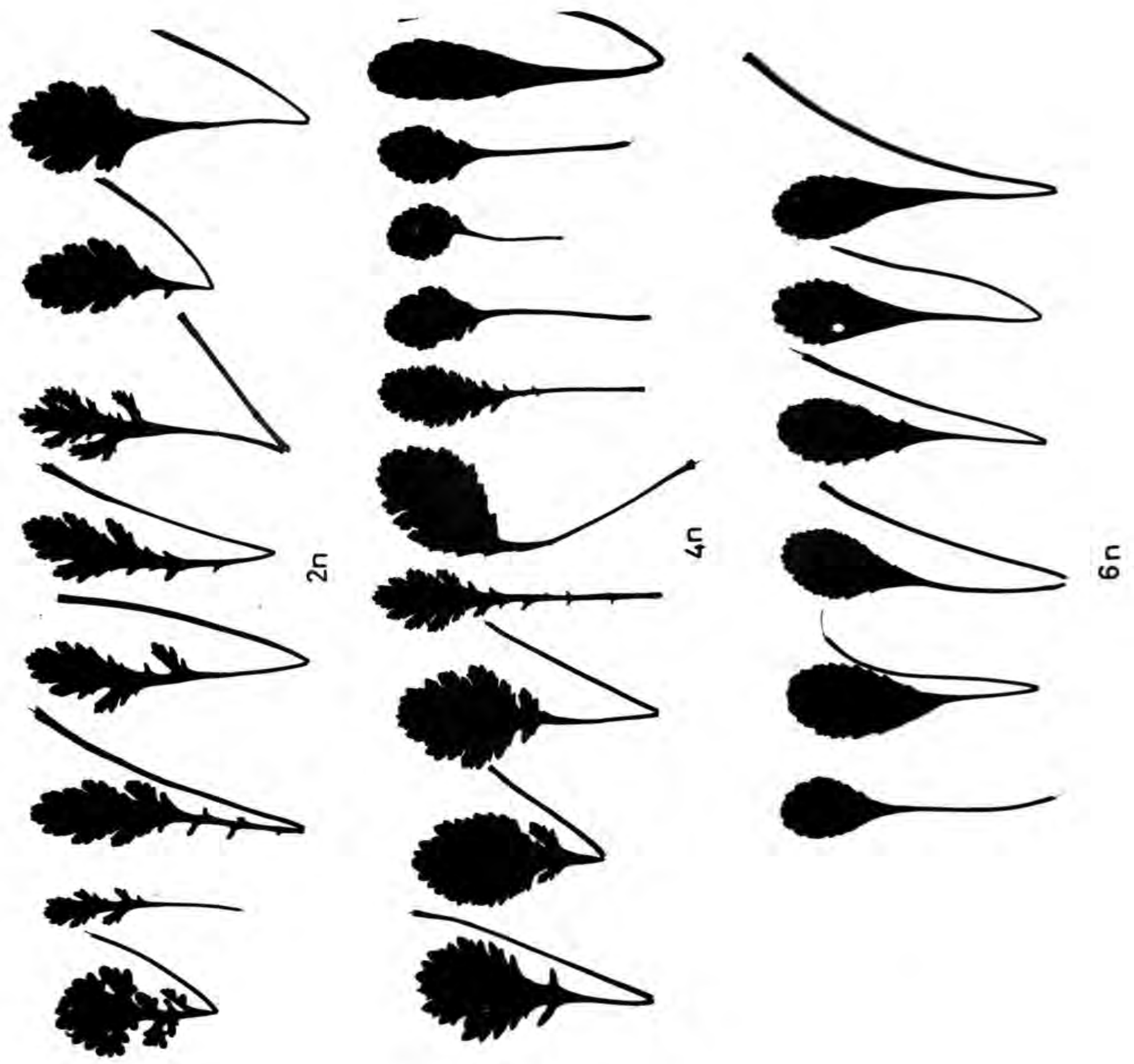


Fig. 56.

unnecessary character weighting, see page 116 .

b) the stem leaf auricles tended to be thinner, longer and frequently more in number in diploids than in the tetraploids. Fig. 55 page 101.

This brief investigation of character variation outlines the following points:-

1) A variety of characters showed interesting tendencies but apparently failed either singly or in simple combinations to give a satisfactory split.

2) The pattern of variation of a series of overlaps could mean that a multivariate approach to the problem might be more fruitful.

Towards this end a suite of computer programs was written and tested. Specifications of the programs and print outs of programs and results can be found in Appendix XIII, page 181A. Fig. 57 page 104 shows the general flow diagram for the suite of programs. The programs are designed to analyse large amounts of data rapidly whilst reducing manual handling of data to an essential minimum.

Material for the analysis was selected from cytologically identified plants and grown on a randomised growth experiment from October 1964 until July 1965 when it was harvested for analysis. The details of the growth treatment are given in Appendix XI page 180 .

Drawing on personal experience and from the literature, 51 characters were selected. It was appreciated that some of the characters selected

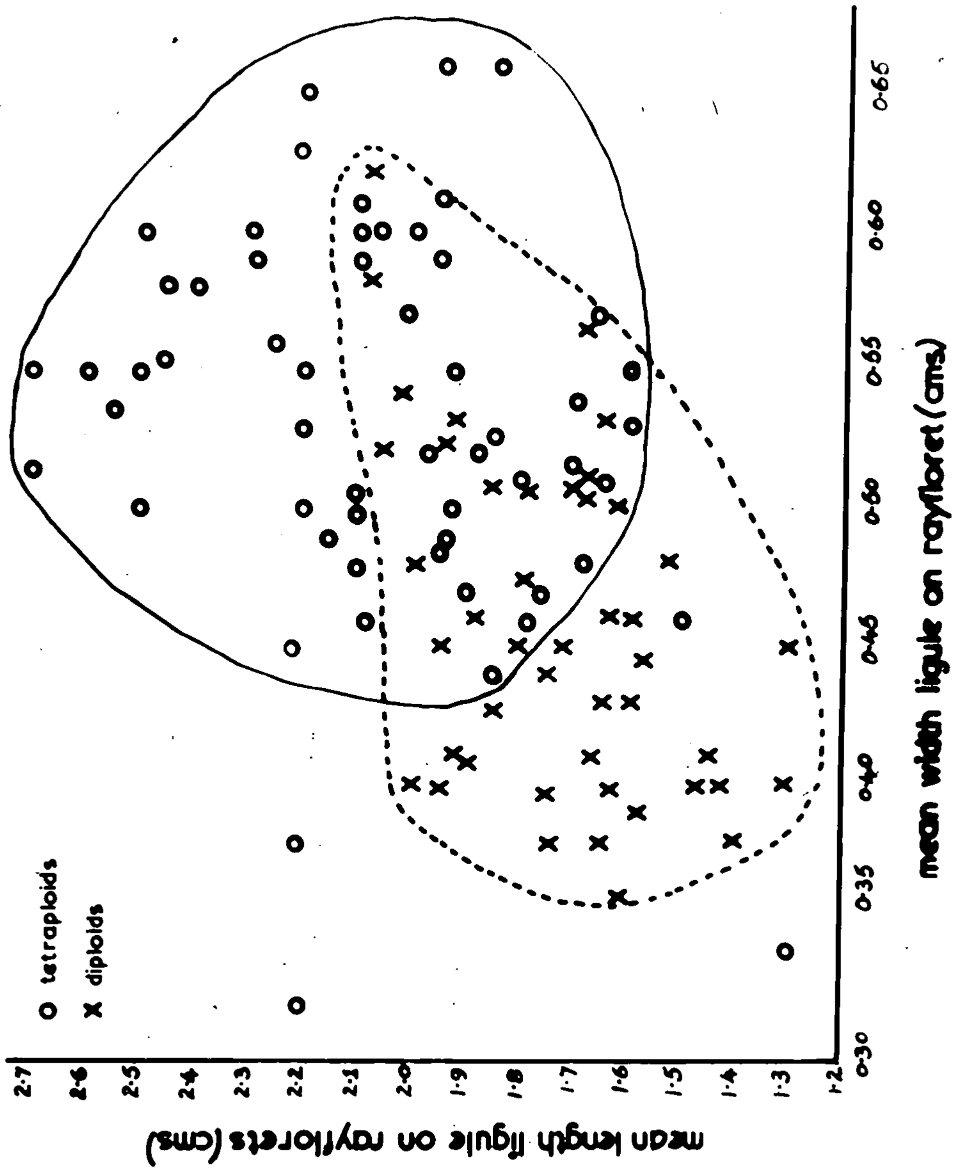
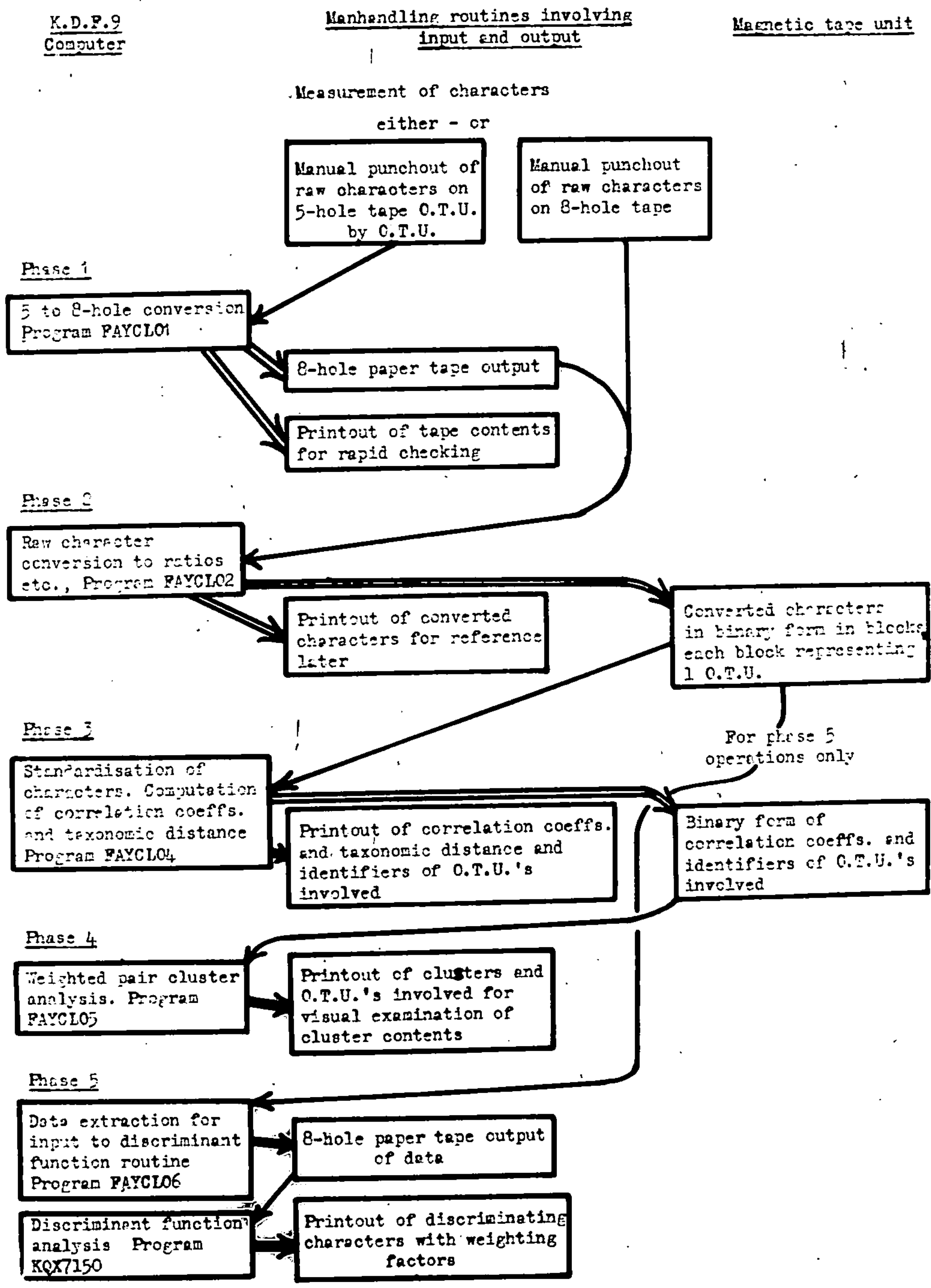


Fig. 56A

Fig. 57

Flow diagram on use of computer programs for multivariate analysis



appeared useless but since they had been used in the literature it was felt that incorporation of them into the analysis might reveal unnoticed correlations. Individual specimens were used for this analysis and not the mean values computed from population samples for three very pertinent reasons:-

1) The basic reference point for the analysis was chromosome count and this had been carried out on individuals which were not necessarily representative of large population samples. The sympatric behaviour of the cytotypes in the British Isles made assumption of chromosome number by virtue of counts having been carried out on other members of a population unjustified.

2) Character variation within a population was so great that a sample large enough to accurately define the various character means would have been prohibitively large in terms of the labour and space available. The problem of how populations fitted into the general scheme of species variation was tackled by including batches of individuals sampled from the same populations.

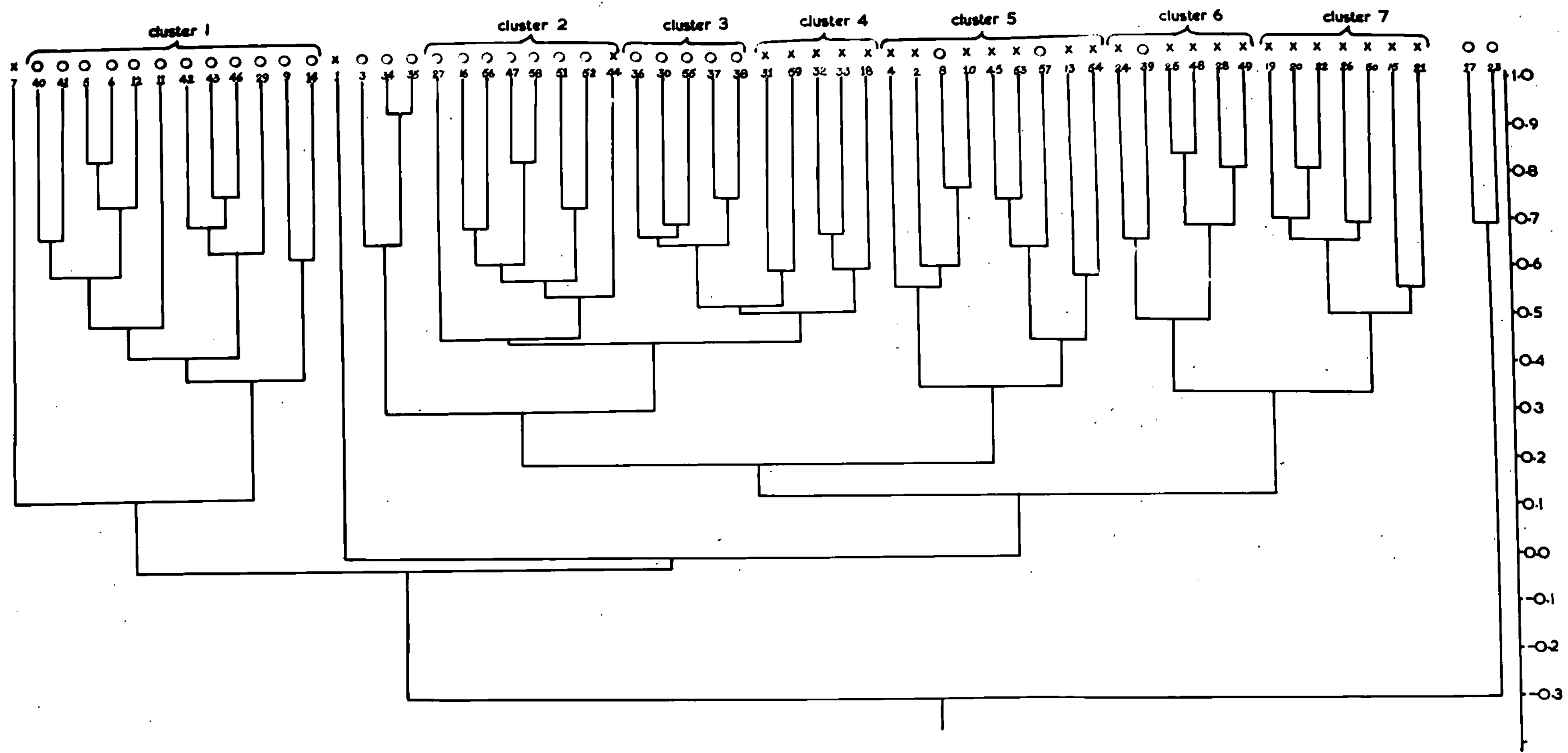
3) Even if use of population mean values was a viable proposition there was no obvious way, other than taking individuals in large numbers from a population, of adequately representing populational character variance in this type of analysis.

The characters used are described in Appendix XIV page 196. Variation of characters within an individual e.g. width of mid stem leaves on

different stems of the same plant, was troublesome and care was taken to measure sufficient replicates from each plant, where they were available, to establish a reliable mean value. As expected, intraplant variation was far less than interplant variation in specimens from the same population and I felt that in the absence of any other procedure, the method of taking character means within individuals was the only suitable one available.

The assumptions made in the multivariate analysis are given in Appendix XII page 181. In the following pages the term operational taxonomic unit (O.T.U.) is used frequently. This term denotes the basic unit which is being classified and in this particular analysis refers to individual plants.

Although data from 140 plants had been collated it was decided to run an initial survey on 60 O.T.U's to see whether there was any point in analysing the whole data. Fig. 58, page 107 shows the results of the cluster analysis of this data. There were some very disturbing features brought out in the analysis, the principal one being that O.T.U's from the same polyploid level and even same population did not cluster into major groups to the exclusions of O.T.U's from other populations and polyploid levels. T-tests were carried out on the differences of transformed within and between cluster correlation coefficient matrices of clusters 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7 and 4 and 7, and with the exception of clusters 2 and 3, were all found to be significantly different to at least the 0.5% level.



CLUSTER ANALYSIS

x = tetraploid o = diploid Origins of OTU's given on page 107a

Fig. 58.

Origins of the O.T.U's used

Locality	Number of O.T.U.
Bearpark, Durham City	14, 16, 30, 36, 37, 38, 39, 42, 55, **29**, 56, 57, 58.
Loggerheads, Flintshire	5, 6, 12, [illegible], 40, 43, 47, 51, 52.
Malham Moor, Yorkshire	10, [illegible] 18, 31, 45, 49, 53, 54, 59.
Strasbourg, France.	15, 19, 20, 21, 22, 26.
Chrysanthemum _rotundifolium_ Czechoslovakia.	17, 23.
Synthesised tetraploids produced from Loggerheads parent plant.	13, 25, 32, 33, 44.
Triploid hybrid	8.
Various British diploids	6, 11, 34, 35, 46.
Various British tetraploids	1, 7, 48.
High Central Pyrenees	41.
Various Continental tetraploids	2, 4, 27, 28, 31, 50, **24**.

This showed that clustering above the +0.45 correlation coefficient level was statistically sound if somewhat biologically curious.

Other interesting facts derived from the cluster analysis include the following:-

A. Two specimens of Chrysanthemum rotundifolium e.g. 17 and 23, were strongly negatively correlated with all the other specimens which were all members of the Chrysanthemum leucanthemum L. species aggregate.

B. Cluster 7 was comprised of individuals taken from Strasbourg and represented a tetraploid morphology frequently found on the Continent but not in the British Isles.

C. A triploid hybrid, O.T.U. 8 was strongly associated with tetraploid plants, which agreed with it having received twice as many genes from the tetraploid parent as the diploid parent.

D. O.T.U's 25, 33 and 32 were synthesised autotetraploids which were strongly associated with natural tetraploids.

O.T.U. 44 was a synthesised autotetraploid strongly associated with diploids.

O.T.U. 13 was a synthesised autotetraploid associated with both diploids and tetraploids.

The autotetraploids were all synthesised from seed taken from a diploid plant from the Loggerheads population.

E. Clusters 6 and 5 were extremely heterogeneous in their contents.

F. The original idea of taking single specimens as O.T.U's was

been vindicated, since the use of population means would have masked the segregation of individuals from the same populations which has occurred.

G. With the exception of cluster 7, which was homogeneous in content, the results did not give any impression of nicely partitioned data between a diploid and tetraploid species, but that one was examining subsets of a single extremely variable taxon in which there was a predominence of diploidy in one sector and of tetraploidy in another.

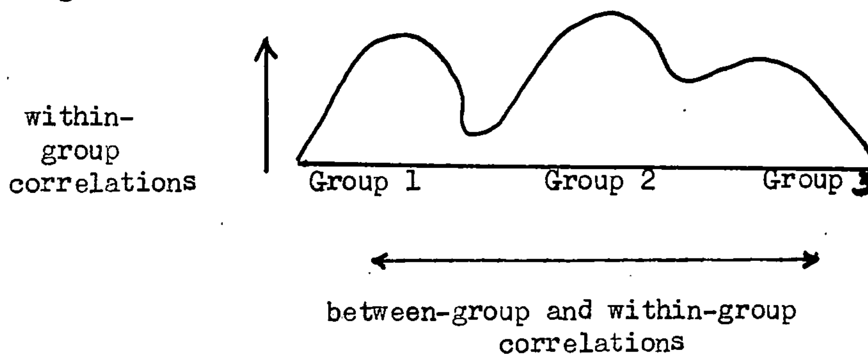
The possibility that the clustering method had distorted the multidimensional relationships between O.T.U's to produce the curious clusters found does not seem reasonable, since the between cluster T-tests were highly significant. The correspondence between the essentially two-dimensional relationships produced by the clustering technique and the multidimensional relationships as indicated by the original correlation coefficient matrix has a correlation of +0.86. This method of assessing the amount of distortion introduced by cluster analysis has been described by Sokal and Rohlf (1962) and named cophenetic analysis. The cophenetic value of +0.86 is as high or higher than those published for other clustering programs. Sokal and Rohlf (1962). The mathematics employed appeared to be satisfactory, so that one must look elsewhere for the reasons for cluster arrangement. I think that there are three possibilities to account for this:-

1. The clustering is a result of some unappreciated character weighting. This does not seem very likely since it is difficult to see how such weighting could result in the clusters shown in Fig. 58 page 107. Casual inspection of the data has not revealed any obvious bias. For example, character variation may have been reflecting the stage of the flowering season at which the plants concerned were harvested for analysis and resulted in clusters of early, middle or late flowering plants. Naturally, for this to have occurred, all or most of the characters would have had to have varied in the same direction relative to time of flowering.

2. The clusters are a true reflection of morphological differences between O.T.U's. If this is the situation then the system is detecting genetical segregation on a grand scale, a statement which is easy to make but less easy to substantiate. The sample sizes involved in this analysis are too small to make anything other than interesting speculations. Within-population correlations extend from values of +0.75 to down to -0.30 and individuals do appear to fall into two or more groups with high within-group correlations and lower between-group correlations. For example, 13 individuals representing the diploid population from Bearpark, Durham, 8 individuals representing the diploid population from Loggerheads, Flintshire, and 8 individuals representing the tetraploid population from Malham Moor, Yorkshire, fall into three groups comprised as follows:-

	Group 1	Group 2	Group 3
Bearpark	29, 42, 14	58, 16, 56, 30, 36, 37, 55, 38	39, 57
Loggerheads	43, 6, 5, 12, 40.	47, 51, 52	-
Malham	-	31, 59, 18, 10	49, 53, 45, 54.

These groups were derived by inspection of the original correlation coefficient matrix and were not taken from the cluster analysis. Certain high between-group correlations tend to blur the discontinuities so that they can perhaps be best represented by the following diagram:-



3. A high proportion of the characters chosen are varying randomly in an uncorrelated fashion and are of no taxonomic value. To some extent this possibility overlaps with the considerations of possibility 1. given above, in that random fluctuations of character variation could give unnecessary weight to certain groups of characters and result in spurious clusterings. I felt that this possibility

could account for some of the anomalies produced by the cluster analysis.

At this stage in the multivariate analysis I had to consider which type of further analysis would give the most important information. The present results did not justify using computer time to work out discriminant characters on such small, heterogeneous clusters. Test runs of the discriminant analysis program on small batches of data had revealed that discrimination was almost totally bound up in chromosome count and pollen grain size when diploid and tetraploid batches were being compared.

In addition I felt that the extension of the present analysis to include data for 140 O.T.U's would give little further information on within-populational variation and that what was required was an investigation in depth rather than width, involving samples from only two or three populations. Up to 70 plants per population could be included so that a detailed analysis of how individual characters were contributing to the within-populational variation could be investigated. Since the material required for such an analysis was not available at the time, I decided to wait for another growing season to collect, grow and harvest the necessary plants.

No research is complete, and this particular research less so than most. It is in this very unsatisfactory stage in the multivariate-analysis that I have had to write this thesis. It is done so from

the position of having developed a powerful method of analysis and with it, detecting a most interesting and unexpected type of morphological variation, which is worthy of further investigation.

Appendix XVI page 205, contains data and results of significance tests of interpopulational character comparisons.

Discussion

It is not my intention to discuss in detail the meaning of, and rationale for classifications resulting from, a multivariate approach, the following texts do this most adequately, Cain and Harrison (1958) and (1960), Michener and Sokal (1958), Sneath and Sokal (1962), Sokal and Sneath (1963), Davis and Heywood (1963), Heywood and McNeill (1964). Suffice it to say, that I think that the value of multivariate methods lies in the way in which more than a few characters can be considered simultaneously in a comparative index, such as the product moment correlation coefficient. Anderson's hybrid index (1936) can conveniently be used for this purpose on small numbers of characters, but is of little value on larger numbers of characters. This is due to the necessity of partioning the variation of each character into an equivalent number of coded units and hence giving a subjective weighting factor. Davis and Heywood (1963), Sokal and Sneath (1963) have erroneously suggested that Anderson's hybrid index is akin to discriminant function analysis. Discriminant function analysis isolates out those characters and character values from a sample of characters which discriminate between two groups of organisms. The process is based

on selecting those characters which minimise the within-group character variance and maximise the between-group character variances, Whitehead, (1954). Whilst it is agreed that this process is also the one which a taxonomist may use intuitively when selecting characters for use in a hybrid index analysis, testing the usefulness of the characters selected is one of trial and error to find the right number and combination of characters which will adequately discriminate between groups. It might perhaps be more logical to regard the hybrid index technique as a rather crude combined index of comparison and clustering technique.

The clusters or taxa derived by the clustering techniques are natural in the sense of being composed of O.T.U's which have an overall similarity based on a maximum number of attributes. There are no phylogenetical connotations attached to such results and whilst phylogenetical reasons, if they were known, may provide an explanation for the arrangement of clusters of O.T.U's, all that can be safely assumed is that clusters have been constructed 'naturally' as defined by Gilmour (1940). Cain and Harrison (1960) have called classifications based upon natural clusters, phenetic, and have defined and illustrated precisely the differences between phenetic and phylogenetic or phyletic classifications.

No taxonomy is better than the characters upon which it is based and this is accentuated in numerical taxonomy where the

large numbers of characters involved can result in large errors from faulty character selection. Unfortunately, the large numbers of characters employed often precludes an intimate knowledge of variation of the characters concerned. Frequently, as has been carried out in the present analysis, other authorities have to be consulted on the availability and type of suitable characters, e.g. Michener and Sokal (1958) used characters which competent taxonomists had thought useful.

Kendrick (1964) has defined a character as any attribute of an organism that can be detected and described. This definition has been refined by Kendrick for taximetric work to the following:- "any attribute feferring to form, structure or behaviour which can occur in any group of organisms as one or more mutually exclusive states". To this should be added "and which cannot be further sub-divided logically", Sokal and Sneath (1963).

Cain and Harrison (1958) have explicitly stated the rationale for choosing characters for taximetric work. It includes considerations of logically correlated characters in which two or more characters are correlated through a functional relationship e.g. an increase of cell size associated with an increase in chromosome number can effect both pollen grain size and guard cell size. It would be unjustified to include both of these characters in a multivariate analysis. Practically, the greatest difficulties arise when characters have partial, logical correlations between them e.g. increase of leaf area is dependant upon

increase of leaf length and if leaf width and area are being used in an analysis, then leaf length would have to be excluded. In addition, invariate characters should not be included in an analysis.

Application of such rigid criteria to the present study has been met with varying success. Some characters had an overall high correlation but were included because the degree of correlation varied amongst the O.T.U's studied. For example, the mean depth of leaf incisions of basal, lower stem and mid stem leaves were correlated positively but the degree of correlation amongst O.T.U's differed. From personal experience of morphological variation within the species complex being studied, I feel that such differences in correlation levels are of taxonomic value. It is probable that they represent genetical differences, since they appear to be fairly constant. A cursory examination of variation of the characters concerned would probably have resulted in assessing that the depth of leaf incisions of different leaves were completely dependant upon each other. The problem of detecting partially dependant characters is a difficulty in which only experience of variation of the characters concerned can be of any assistance. My own experience would indicate that the number of partially dependant characters recognised is more or less proportional to the amount of time spent studying variation of the plants concerned.

Partial dependancy of characters produces a partial weighting which cannot be avoided, but complete dependancy can and should be

avoided. Sarker et al. (1966), working on soil types, recommend carrying out an initial R-type correlation coefficient and cluster analysis between characters from the same potential soil type and eliminating all but one member of groups of similar characteristics produced by high correlation coefficients. The remaining characters, which should then vary independantly of each other, can be used in a normal Q-type analysis, see page 181. A problem in using this technique lies in the danger of eliminating partially dependant characters, where the independant variation is of taxonomic interest. The only logical procedure would be to select a level of correlation above which characters could be regarded as being fully dependant, i.e. where the correlation is not significantly different from a value of +1 or -1. Normal confidence limits based upon such observations would set the correlation levels at approximately -.75 and +.75, respectively. However, the precise levels would have to be determined by trial and error using partially dependant characters, whose taxonomic worth had already been assessed by other methods.

Most numerical taxonomists support the idea of giving equal weight to characters on the grounds that there are no precise criteria for weighting characters. Kendrick and Proctor (1964) have advocated giving differential weighting on the basis of absence of 'primary' characters which can be subdivided into a number of 'secondary' characters. They think that differences between two O.T.U's, one of which lacks a 'primary' character, should express not one character

difference but x character differences, where x represents the number of 'secondary' characters present in the primary character concerned. An example of a 'primary' character and its associated 'secondary' characters might be a stem hair and its size and shape parameters. Long (1966) has pointed out correctly the inherent errors in proposing such a weighting system and that it would not have resulted if Kendrick and Proctor had had an accurate understanding of the definition of a character as used by most numerical taxonomists. Their defect appears to lie in disregard for the phrase "and which cannot be further subdivided logically", (page 116).

Similarly, Whitehead (1954) recommended weighting characters proportionally to their discriminant function. The error in logic behind this particular weighting technique, or indeed that suggested by Williams et al. (1964) lies in the necessity to first circumscribe the groups. There is no obvious way around this particular problem.

Williams and Lance (1965) have pointed out that since polythetic classifications are normally based upon a finite number of individuals and attributes, they are probabilistic. Splits and fusions of groups within such a classification only have a probability and not actuality of reflecting a biological situation. They add that since, at least theoretically, the uses of a polythetic classification are infinite and the numbers of individuals and attributes considered finite, then this produces difficulties in assigning a probability or significance level to the fusion or splitting of groups. Williams and Lance suggest

that it is more logical to consider classifications not on their probabilities but on whether they can be assessed as profitable or informative. From this it follows that there are no inherently 'correct' classifications and the argument that polythetic classifications are better because they contain more information, becomes redundant if information can be extracted more efficiently from monothetic classifications.

However, if classifications have a predictive or useful value then this value must be a reflection not only of the number of attributes and individuals utilised in constructing the classification, but also of the 'quality' of those attributes or characters.

In the present study, although some interesting morphological correlations have emerged, insufficient knowledge was known about the characters prior to the analysis. It is inadequate to accept on faith morphological characters which previously herbarium sheet taxonomists have used, or which one intuitively feels are of taxonomic importance. Only from growth, genetical and comparative morphological experiments can the necessary information for adequate character selection be collated. Unfortunately, the morphological characters which plant taxonomists use normally have complex developmental and variational patterns, which can only be adequately described after intensive study. The enormity of such a task precludes the possibility of carrying out an analysis on a wide range of different taxa successfully. Because of this I feel that numerical taxonomical methods can probably be applied to the best advantage to studies in depth of taxonomic groups at or

beneath the generic level since it is only in such a situation that the many facets of character variation can be studied adequately.

Conclusions

Morphological variation in Chrysanthemum leucanthemum L. has proved most difficult to dichotomise logically. Single characters or simple combinations of characters have been shown to be inadequate to fully distinguish between diploid and tetraploid population samples.

Initial analyses by numerical taxonomic methods have indicated the need for intensive investigations on intra-populational variation before a morphological survey of the species complex can be adequately carried out.

SECTION VI

TAXONOMICAL AND NOMENCLATURAL CONSIDERATIONS IN *CHRYSANTHEMUM LEUCANTHEMUM* L. *SENSU LATO*

TAXONOMICAL AND NOMENCLATURAL CONSIDERATIONS IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

From a position of having incomplete information on morphological variation, it is difficult to make meaningful recommendations on the taxonomy of a group. It might be useful to briefly consider what information has been gained from the present and previous studies and compare it to what would be required for a full taxononomic revision of Chrysanthemum leucanthemum L. sensu lato.

The following are the most important points:-

1) 85% of the material examined under experimental conditions was of British origin. This bias means that comments passed on material of foreign origin could be subject to enormous sampling errors.

2) The environmental contribution to morphological variation is probably considerable. This means that a taxonomic study carried out solely from herbarium specimens could result in serious errors in making reliable assessments of morphological similarities and dissimilarities. In other words, material gathered from wide ranging localities needs to be grown under uniform conditions on a much wider scale than has been attempted to date in Chrysanthemum leucanthemum L. Another, and perhaps more feasible alternative would be to collate the experimental data of several different workers from different countries. By this it would not be intended simply to list chromosome numbers, as has already been carried out ad nauseam, but to make voucher specimens complete with details of growth conditions, fully available.

3) There appears to be insufficient evidence for regarding British material as two species, corresponding to the diploid and tetraploid morphological levels of polyploidy. This judgement must be tempered by the fact that complexities of morphological variation within British populations require further analysis.

4) The recent paper of Favarger and Villard (1966) indicates that the morphological criteria for separating diploids and tetraploids in Swiss material may be far flimsier than the prior paper of Favarger (1959) led one to believe. Using the varietal nomenclature of Briquet et Cavillier, they state:-

"il y a aussi des plantes à morphologie intermédiaire entre celle de la var. lobatum (diploide) et celle de la var. pratense (tétraploide), par exemple des individus dont la feuille, plus large que dans la première, l'est moins toutefois que dans la seconde". From this, and other points in their paper, it appears that the varieties lobatum (diploid) and pratense (tetraploid) (see page 125) grow together, have morphological intermediates between then and can only be distinguished by a difference in flowering time. They state that diploids commence to flower 20 days before the tetraploids.* A most disturbing feature of the above-mentioned paper is that the lists of chromosome counts

---

* In the present work growth experiments revealed no overall differences in time of flowering of diploids and tetraploids of British and Continental plants. However, induction of polyploidy by colchicine treatment, cause the synthesised polyploids to flower some days before the parent diploids.

reveal that less than 110 diploid and tetraploid plants have been counted.

Bearing in mind the incomplete state of knowledge of variation in the species both from my own work and from others, I have very tentatively constructed a working check-list and a set of descriptions page 124. No attempt has been made to construct an artificial key.

Chrysanthemum leucanthemum was first described by Linneaus in his Hortus Cliffortianus and later in Species Plantarum. Lamark in Flora Francaise transferred the species to the genus Leucanthemum Mill. and gave it the specific name of vulgare. Since the time of Lamark, various authors have placed the 'leucanthemum' species into either of the two genera. Briquet et Cavillier (1916), employing both anatomical and carpological evidence, supported the view of Lamark. Since then the embryological work of Harling (1951) has added further evidence for the acceptance of Chrysanthemum, Leucanthemum and Tanacetum as separate genera. The classification in the British Flora appears to have been governed by the illogical position adopted by Bentham and Hooker, (Heywood, (1958)), who united Pyrethrum and Leucanthemum with Chrysanthemum but kept Tanacetum as a separate genus. The latest edition of Clapham, Tutin and Warburgh (1962) recognises Chrysanthemum, Leucanthemum and Tanacetum as subgenera of the genus Chrysanthemum.

Up to this point in the script, the generic name of Chrysanthemum has been used for personal convenience, but in the following examination

of specific nomenclature Leucanthemum will be regarded as the valid generic name.

A nomenclatural check-list of Leucanthemum vulgare Lamk.

Leucanthemum vulgare Lamk. Fl. fr. II, 137 (1778)

= Chrysanthemum leucanthemum L.

Sp. Plant., Ed. I, 888 (1753)

Subsp. triviale (Gaudin) Briquet P.P. Fl. Mar. Alp., II, 84 (1916)

= subsp. praecox Hovatic pp. maxima

Acta Bot. Inst. Bot. Un. Zagreb 10, 61, (1935)

= var. pinnatifidum Lec. et Lam.

Cat. pl. vasc. plat. centr., 227 (1847)

Var. alpicolum Gremli Fl. anal. suisse, 2nd Ed., 272, (1898)

= Chrysanthemum atratum Gaud. A. helv. V, 344 (1829).

Var. lobatum Briquet. Ann. cons. et Jard. bot. Geneve, III, 120, (1899)

Var. autumnale (St.Am) Briquet Fl. Mar. Alp., II, 84 (1916).

= var. laciniatum Briq.

Ann. Cons. et Jard. bot. Geneve III, 121 (1899)

= var. coronopifolium Fiori et Paol.

Fl. anal. It., III, 239 (1903)

Subsp. ircutianum (DC) Pearson.

= Leucanthemum ircutianum (Turc=) DC.

Prodr., VI, 46 (1837).

Var. pratense Timb-Laqr. Bull. soc. damph., 1, 230 (1879)

Var. praestans Briquet Fl. Mar. Alp., II, 89 (1916)

The related taxa of Leucanthemum montanum (Allioni), Leucanthemum pallens Rouy, Leucanthemum maximum DC., and Leucanthemum heterophyllum DC have not been considered since insufficient experimental material has been examined. However, tentatively, there appear to be inadequate morphological reasons for regarding them all as separate species. It is possible to trace a continuum of variation of leaf morphology from subsp. triviale to Leucanthemum montanum DC. See fig.56 page 101A.

Descriptions of the subspecies and varieties believed to occur in the British Isles

Leucanthemum vulgare Lamk.

subsp. triviale (Gaudin) Briquet

1. Both branched and unbranched forms
   Less strongly branched than in subsp. ircutianum
   Average number of branches = 0.7 (0-0.7-2) per stem

2. Average height = 46 cms (35-46-65)

3. Average diameter of capitulum (including ray florets) = 5.20 cms (40-5.2-6.8)

4. Average length of ray florets = 1.67 cms (1.45-1.67-2.1)

5. Average diameter of pollen (including spines) = 30.0 microns

6. Number of chromosomes = 18 (28-30-32)

7. Basal leaves irregularly crenately lobed, often deeply incised.

8. Mid-stem leaves linear-lanceolate. Usually irregularly toothed or lobed.

9. Mid-stem leaves usually pinnatifid at base.

10. Both long and short lived perennials.

Var. alpicolum Gremli

L. Average height = 40 cms.

2. Uniformly unbranched

3. Mid-stem leaves are regularly dentate and have long basal auricles.

4. The margins of the involucral bracts are black.

5. This variety is ecologically distinct from other varieties and is found on base rich soils in 'natural' communities e.g. Malham Tarn, Humphrey Head.

Var. lobatum Briquet

1. Average height = 50 cms.

2. Both branched and unbranched forms occur.

3. Basal and mid-stem leaves are small, and irregularly lobed.

4. Basal leaves are obovate and mid-stem leaves lanceolate.

5. The margins of the involucral bracts are dark brown.

6. This is a cosmopolitan variety and grows on roadsides, railway embankments, in meadows and hayfields.

Var. autumnale St. Am.

1. Average height 60 cms.

2. Normally branched.

3. The leaves are large and irregularly and deeply lacinate to pinnati-lobed.

4. The basal leaves are obovate and the mid-stem leaves lanceolate-oblong.

5. The margins of the involucral bracts are dark brown.

6. The stem is frequently of a much lighter green than in other varieties and is usually glabrous.

7. This variety growns on sea cliffs and roadside verges.

Subsp. <u>ircutianum</u> (DC)

1. Both branched and unbranched forms.
   Average number of branches per stem = 1.4 (0-1.4-5)

2. Average height = 54 cms (40-54-75)

3. Average diameter of capitulum (including ray florets) = 5.70 cms (4.5-5.70-7.0)

4. Average length of ray florets = 1.82 cms. (1.52-1.82-2.2)

5. Average diameter of pollen = 340 microns (30-34-37) (including spines)

6. Number of chromosomes = 36

7. Basal leaves more regularly crenate-dentate than in subsp. <u>triviale</u>, normally with no deep incisions.

8. Mid-stem leaves linear-lanceolate to oblanceolate. Both regularly and irregularly toothed.

10. Mid-stem leaves pinnatifid to not pinnatifid at the base.*

11. Usually long lived perennials.

Var. <u>pratense</u> Timb-Laqr

1. Average height = 50 cms.

2. Normally unbranched

3. Basal and stem leaves regularly crenate to dentate.

4. The cauline leaves are obovate to spathulate, narrow towards their bases, and then expand to become obviously auriculate.

---

* All British forms studied have proved to be pinnatifid.

5. This variety grows mainly in hay fields and at the edge of woods.

Var. praestans

1. Average height = 60 cms.

2. Usually branched

3. The leaves are large and often irregularly dentate to crenate, although less so than in the subsp. triviale.

4. The cauline leaves are usually oblong and have much thicker auricles than in the other varieties.

5. This variety grows mainly on road side verges and railway embankments.

To take account of the morphology of all tetraploid plants examined another variety is required and from the descriptions in the literature this appears to correspond to the var. adustum (Koch) Hayek of the subsp. montanum (All) Gaudin. Favarger (1959) regards the 'montanum' taxon as being taxonomically distinct from any of the Leucanthemum vulgare DC. varieties. However, just as Böcher and Larsen (1957) found difficulties in distinguishing Leucanthemum ircutianum DC from Leucanthemum montanum (All.) , I have found difficulties in rationalising a split between the subsp. ircutianum (DC) and the subsp. montanum (All.) Gaudin. The problem should be deferred until further experimental evidence has been obtained.

SECTION VII

GENERAL DISCUSSION

GENERAL DISCUSSION

The different types of examination used in the present study have, in the main, indicated that there are both genetical similarities and dissimilarities between diploid and tetraploid populations. There are several possible explanations for this, including:-

a) Tetraploidy has arisen by 'autopolyploidy', possibly polytopically, and then subsequent hybridisation and evolutionary divergence have resulted in some genetical dissimilarities. This theory is supported by the cytological situation found in newly-synthesised autoploids.

b) Tetraploidy has arisen by hybridisation of diploid species and then induction of 'allopolyploidy' or segmental allopolyploidy (Stebbins, 1947), by chromosome doubling. Favarger & Villard (1966) have shown that the chromosomes of diploid Chrysanthemum leucanthemum L. and Chrysanthemum atratum Jacq., pair at meiosis in the synthesised hybrid which, however, is sterile. They did not induce an 'allopolyploid' from the hybrid, which would have been a most interesting and valuable experiment to perform.

c) Tetraploidy has arisen by a mixture of processes (a) and (b). In an outbreeding species such as Chrysanthemum leucanthemum L. true autopolyploidy can never arise even by straight chromosome doubling since there is always a large amount of heterozygosity in diploid populations. Similarly, when members of different populations of the same species or of different, closely related species, hybridise, the hybrids are unlikely to be completely heterozygous at all loci.

Induction of polyploidy will, therefore, rarely result in true auto or allopolyploidy, in outbreeding species. A basic misconception, perpetuated by plant biosystematists, is to refer to polyploidy arising by hybridisation between different species and then chromosome doubling as allopolyploid. The term allopolyploid is a genetical one and should not be directly linked with morphological species, since the genetical similarities and dissimilarities between species are often unknown and can only be assessed by genetical analysis.

The problem of incorporating genetical, evolutionary or indeed any type of information other than purely morphological, into the framework of a classical taxonomic classification is a general dilemma which can only be resolved by considering each case on its merits. There are no inherently right or wrong classifications, since classifications are conceptions of the human mind to assist in describing and cataloguing information in a readily retreivable form. Species, Genera, Families etc. are abstractions which are created to describe biological situations in which groups of organisms are distinct from one another in certain attributes. Although traditionally the attributes considered have been purely morphological, frequently with <u>a priori</u> reasoning on the importance of such attributes thrown in for good measure, from the above reasoning it follows that there are as many possible types of species as there are types of attribute.

The 'raison d'être' for the biological situation approximated by the morphological species is the breeding unit.* The breeding

* It is a not unreasonable assumption that all groups of higher organisms, including flowering plants, were outbreeding at some stage of their evolution and that this preceeded inbreeding systems.

unit reflects one of a number of different isolating mechanisms including spatial, temporal, structural and genetical, Davis and Heywood (1963), and has the potentiality for individual evolution or speciation.

Valentine (1949) has made the distinction between isolating mechanisms which evolve gradually and those which are produced abruptly. Induction of polyploidy is an example of an 'abrupt' isolating mechanism. It is accepted that in general a change in level of ploidy constitutes a barrier against gene flow between the two ploidy levels. In most instances, the formation of triploids does not initiate gene flow between the diploid and tetraploid levels. However, in situations in which diploids and tetraploids can be sympatric as in the present study, there is always the possibility of fertilisation involving unreduced gametes from diploid plants and normal gametes from tetraploid plants.* Such hybridisation would be unidirectional.

Consideration of evolutionary mechanisms and pathways is an intriguing pursuit but unfortunately has no direct contribution to make to classical taxonomy. However, knowledge of breeding groups may be of assistance for indicating where to look for morphological variations which can be given taxonomic recognition. Where morphological and genetical discontinuities coincide then natural

---

* In the hybridisation experiments described in Section III, some tetraploid plants resulting from 2n x 4n crosses, and originally interpreted as being produced by tetraploid selfing were found to be highly sterile. These could have been hybrids produced from unreduced diploid male gametes and tetraploid female gametes.

taxonomic groups can be created. Some biosystematists regard the possession of different levels of polyploidy within an existing species as sufficient evidence for creating separate species.

For the general requirements of taxonomists only morphologically different groups of plants have a usefulness as separate taxa and "cryptic polyploids should, therefore, receive no taxonomic recognition." Heywood, (1959). Walters (1962) has pointed out that experimentalists have to use the framework of orthodox taxonomy as basic reference points. The usefulness of such a morphological framework would be decreased if it were unduly distorted by trying to incorporate genetical and other types of experimental data. The usefulness of the framework would also be increased if such information could be incorporated without distortion. Various systems of classifying experimental information and using them to supplement the classifications of orthodox taxonomy have been proposed. These include the gene exchange system of Turesson (1929) and the multi-purpose Deme system proposed by Gilmour and Gregor (1939). The benefits and difficulties of applying such systems have been discussed by Valentine and Löve (1958), and Davis and Heywood (1963). It might be added that the necessity for using biosystematic classifications has never been felt in the present study.

With regard to the present study, there appear to be insufficient morphological differentiae for successfully dichotomising British oxe-eyed daisies into groups corresponding to the diploid and tetraploid

states. There are some interesting parallels between this work and that of Torres (1965) on the chromosome races of *Zinnia juniperifolia* who found tetraploids with a reduced quadrivalent frequency below the expected value for observed chiasma frequency in diploids. In addition there were a few rather indiscriminate morphological differences between the chromosome races. The closing remarks of Torres are equally applicable to the *Chrysanthemum leucanthemum L.* situation in the British Isles:- "At this point it seems best, at least tentatively, to retain the tetraploid as a race of the parent species."

APPENDIX I

A LIST OF CHROMOSOME COUNTS AND THE ORIGIN OF THE PLANTS USED IN THE PRESENT WORK

APPENDIX I

Origin of material	Ecological feature of locality	Count	No. of plants counted
Festiniog, Merionethshire	Hayfield	2n = 18 n = 9	10
Aberdovey, Merionethshire	Roadside - grass verge	2n = 18 n = 9	3
Pantymyn, Denbighshire	Limestone bank, roadside	2n = 36	3
Corwen, Merionethshire	Grazed pasture-river bank	2n = 18 n = 9	3
Loggerheads, Flintshire	Limestone quarry bank	2n = 18	5
Aberdovey, Merionethshire	Cemetery, mown grassland	2n = 18 n = 9	5
Aberystwyth, Cardiganshire	Botanic Gardens	2n = 36	1
Maeshafn, Flintshire	Hayfield	2n = 18	5
Gwynermynedd, Flintshire	Roadside, mown grass verge	2n = 18	10
Loggerheads, Flintshire	Limestone cliff	2n = 18	5
Cwym Idwal, Caernarvonshire	Rock face, 2,000 ft.	2n = 18	10
Carnedd Daffydd, Caernarvonshire	Wet rock ledges, 2,500 ft.	2n = 18	5
Little Haven, Pembrokeshire	Sea cliffs	2n = 18 n = 9	10
Monkhaven, Pembrokeshire	Sea cliffs	2n = 18	1
Par Sands, Cornwall	Boulder clay, sea cliff	2n = 18 n = 9	1

Origin of material	Ecological feature of locality	Count	No. of plants counted
Bexhill-on-sea, Sussex	Permanent pasture	2n = 36	3
Bedford, Bedfordshire	Bank of river	2n = 36	10
Fen End, Warwickshire	Grass verge in cemetery	2n = 36 n = 18	10
Matlock, Derbyshire	Pasture, river bank	2n = 36 n = 18	10
Buxton, Derbyshire	Limestone bank, roadside	2n = 36 n = 18	10
Edale, Derbyshire	Edge of meadow, grazed	2n = 36 n = 18	10
East Yorkshire	Limestone bank, roadside	2n = 36	2
Malham Moor, Yorkshire	Roadside verge	2n = 36 n = 18	20
Malham Tarn, Yorkshire	Limestone rock ledges, 1,300 ft.	2n = 18 n = 9	3
Hellifield, Yorkshire	Railway embankment	2n = 36	1
Teesdale, Yorkshire	Dry water course	2n = 36	3
Teesdale, Yorkshire	Grazed permanent pasture	2n = 36	6
Pickering, Yorkshire	Stubble cornfield	2n = 36	6
Millersdale, Derbyshire	Bed of limestone quarry	2n = 36	6
Cassop Vale, Co. Durham	Pit slag heap	2n = 36	3

Origin of material	Ecological feature of locality	Count	No. of plants counted
Ferryhill, Co. Durham	Permanent pasture	$2n = 18$	1
Durham City, Co. Durham	Roadside bank	$2n = 18$ $n = 9$	10
Durham City, Co. Durham	Disused openland	$2n = 36$ $n = 18$	5
Silverdale, Westmorland	Limestone bank, roadside	$2n = 36$	1
Shap, Westmorland	Unknown roadside verge	$2n = 36$ $n = 18$	10
Lawyers, Perthshire	Hayfield	$2n = 36$	3
Enochdhu, Perthshire	Waste ground, roadside	$2n = 36$	3
Ballinling, Perthshire	Permanent pasture	$2n = 36$	3
Pitlochry, Perthshire	Roadside grass verge	$2n = 36$	1
Keltny Burn, Perthshire	Roadside grass verge	$2n = 36$	10
Fort William, Invernesshire	Roadside grass verge	$2n = 36$	3
Inverness, Invernesshire	Roadside grass verge	$2n = 36$	3
Old Meldrum, Aberdeenshire	Roadside grass verge	$2n = 36$	10
Broadford, Skye	Permanent pasture	$2n = 36$ $n = 18$	3
Dornock Firth, Sutherland	Roadside grass verge	$2n = 36$	1
Sligo, Eire	Roadside grass verge	$2n = 36$	1

Origin of material	Ecological feature of locality	Count	No. of plants counted
Lizard, Cornwall	Sea cliff	2n = 18 n = 9	10
Various localities, Cornwall	Sea cliff	2n = 18	30
Friuli, Italy	Not known	2n = 36	3
San Pelegrino, Italy	Limestone, 2,000 ft.	2n = 36	3
Udine, Italy	Not known	2n = 36 n = 18	8
Ispragunaja, Caucassus, USSR	Not known	2n = 36	3
Leningrad, USSR	Not known	2n = 54 n = 18	1
Minsk, USSR	Not known	2n = 36	1
Bohemia centralis, CSSR (1)	Not known	2n = 36 n = 18	3
Bohemia centralis, CSSR (2)	Not known	2n = 36 n = 18	3
Bohemia centralis, CSSR	Not known	2n = 36 n = 18	8
Moravia septentrion-alis, CSSR	Not known	2n = 36	8
Bohemia centralis, CSSR	Not known	2n = 36	10
Carparticum, Praetatricum, CSSR	Not known	2n = 36	3
Slovakia septen-trionalis [illegible], [illegible]	Not known [illegible]	2n = 36	3

Origin of material	Ecological feature of locality	Count	No. of plants counted
Liberec, CSSR	Mountain side	2n = 36	1
Sturovo, Slovakia meridionalis CSSR	Sandy habitat, Bank of Danube	2n = 36 n = 18	5
Wolin Insula, Poland	Not known	2n = 36	3
Bakia-Gora, Cracow, Poland (1)	Mountain side	2n = 36	3
Cracow, Poland (2)	Mountain side	2n = 36	3
Komapolsa, CCCP	Not known	2n = 36	3
Maria besuyo, Hungary	Not known	2n = 36	3
Zagreb, Yugoslavia	Not known	2n = 36	3
Sarajevo, Yugoslavia	High meadow, 5,000 ft.	2n = 18	2
Sott d'Sella, France	Not known	2n = 36	1
Kircheim, Austria	Not known	2n = 36	2
Crozon, Finistere, France	Not known	2n = 18	10
Lauteret, Haute Alpe, France	Not known	2n = 54	10
Nantes, France	Not known	2n = 36	3
Massif du Hohneck, Strasbourg, France	Acid meadow, 3,500 ft.	2n = 36	3

Origin of material	Ecological feature of locality	Count	No. of plants counted
Benasque, Central Pyrenees, Spain	Hayfield, 3,000 ft.	2n = 36	8
Valle d'Astos, Central Pyrenees, Spain	Subalpine - pine zone, 4,500 ft.	2n = 18	10
Trou de Toro, Central Pyrenees, Spain	Alpine limestone substratum 7,000 ft.	2n = 18	5
Jaca, Central Pyrenees	Dry pasture, 2,000 ft.	2n = 54	3
Baleia Combra, Portugal	Not known	2n = 54	3
Lund, Sweden	Not known	2n = 36	3
Sibjansnas, Salarma, Sweden	Not known	2n = 36	3
Frederikessund, N.Zealand, Denmark	Sea cliffs	2n = 18	3
Ottawa, Canada	Not known	2n = 18	1
Ottawa, Canada	Not known	2n = 18	1

APPENDIX II

A LIST OF CHROMOSOME COUNTS AND ORIGINS OF THE MATERIAL USED IN PREVIOUSLY PUBLISHED WORKS

APPENDIX II

Summary of Published Chromosome Counts of *Leucanthemum vulgare* Lamk. *sensu lato*

Author	Year	Origin of material	Chrom. Count	Nomenclature given by authors
Tahara	1915	Unknown	2n = 36	*C. leucanthemum* L.
Tahara	1921	Unknown	2n = 36	"
Orth	1950	Central Europe	2n = 36	"
Shimotomai	1933	Unknown	2n = 36	"
Shimotomai	1937	Innsbruck and Kaiserstuhl	2n = 36	*C. ircutianum* Turcz.
Cooper and Mahony	1935	Campus, Univ. of Wisconsin, U.S.A.	n = 18	*C. leucanthemum* L.
Negodi	1937	Unknown	2n = 36	"
Rohweder	1937	Sand-dune region Darr-Zingst, Germany	2n = 36	"
Polya	1950	Railway embankment Province Hajdn-Bihar, Hungary	2n = 18	"
Dowrick	1952	Botanic Gardens Glasnevin, Dublin	2n = 18	"
Dowrick	1952	Botanic Inst. of Lausanne, Switzerland	2n = 54	"
Dowrick	1952	N. Pyrennees	2n = 90	*C. maximum* Ram.
Dowrick	1952	Portugal	2n = 198	*C. lacustre* Brotero
Suzuka	1953	Unknown	2n = 18	*C. leucanthemum* L.
Martin and Smith	1955	Corvallis, Oregon, U.S.A.	2n = 18	"
Löve and Löve	1956	Iceland	2n = 35	"
Baksay	1956	Csokako, Verteskozma Somhohegy, Mts. Vertes, Hungary	2n = 54 2n = 54	*C. maximum* Ram. *C. maximum* Ram. subsp. montanum (Gaudin) amend. Baksay

Author	Year Pub.	Origin of material	Chrom. count	Nomenclature given by authors
Böcher and Larsen	1957	Edenderry and Scraw bog, Eire	2n = 18	C. leucanthemum L. sensu stricto
Böcher and Larsen	1957	Puszczykowo, (Poznan) Poland	2n = 18	" "
Böcher and Larsen	1957	5 localities from Denmark	2n = 18	" "
Böcher and Larsen	1957	Col de Babourde a Quillan, France	2n = 36	C. ircutianum Turcz. or C. montanum All.
Böcher and Larsen	1957	Col de Pillon, Switzerland	2n = 36	"
Böcher and Larsen	1957	Tatra, Poland	2n = 36	"
Böcher and Larsen	1957	Bydgozcz, Poland	2n = 36	"
Böcher and Larsen	1957	3 localities, Yugoslavia	2n = 36	"
Böcher and Larsen	1957	Zagorsk, USSR	2n = 36	"
Böcher and Larsen	1957	6 localities, Sweden	2n = 36	"
Böcher and Larsen	1957	22 localities, Denmark	2n = 36	"
Böcher and Larsen	1957	Porto, Portugal	2n = 54	C. pallens Gay
Baksay	1957	Central Mts. Hungary	2n = 18	C. leucanthemum L. subsp. triviale Gaudin
Baksay	1957	Central Mts. Hungary	2n = 54	C. maximum Ram.
Baksay	1957	High Tatra, Czechslovakia	2n = 54	"
Baksay	1957	High Tatra, Czechslovakia	2n = 54	C. maximum Ram. subsp. montanum (All.) Gaudin amend Baksay.

Author	Year Pub.	Origin of material	Chrom. count	Nomenclature given by authors
Mulligan	1958	Batiscom, Lauzon, Lennozville-Quebec Tidehead New Brunswick	2n = 36	C. leucanthemum L.
Mulligan	1958	32 localities, Newfoundland, Lab., P.E.I., N.S., N.B.? Que., Ontario, B.C. and Me.	2n = 18	C. leucanthemum L var. subpinnatifidum Fernald.
Favarger	1959	Several localities in Switzerland & France	2n = 18	C. leucanthemum L. subsp. praecox Horv. and alpicola Gremli.
Favarger	1959	"	2n = 36	C. ircutianum Turcz.
Favarger	1959	Several localities in Switzerland	2n = 54	C. montanum All.
Favarger	1959	Yugoslavia and N. Italy	2n = 72	C. heterophyllum Willd.
Mulligan	1959	Botanic Garden, Besancon, France	2n = 36	C. leucanthemum L.
Mulligan	1959	Botanic Garden, Moscow	2n = 36	"
Mulligan	1959	Botanic Garden, Porto, Portugal	2n = 54	L. vulgare Hill subsp. crassifolium Hoff. and Link.
Mulligan	1959	"	2n = 54	L. vulgare Hill subsp. pallens D.C.
Skalinska et al	1961	Several localities, Poland	2n = 18 2n = 36 2n = 54	C. leucanthemum L.
Skalinska et al	1964	High Tatra, Poland	2n = 18	"
Skalinska et al	1964	Lowland, Poland	2n = 36	"
Favarger & Villard	1966	Numerous localities, both alpine & lowland in Switzerland	2n = 18 n = 9	C. leucanthemum L. subsp. triviale vars. alpicolum autumnale and lobatum.

Author	Year Pub.	Origin of material	Chrom. count	Nomenclature given by authors
Favarger & Villard	1966	Numerous localities in Switzerland, mainly lowland	2n = 36 n = 18	C. leucanthemum L. subsp. triviale vars. pratense and praestans. Corresponds to C. ircutianum Turcz.
Favarger & Villard	1966	Numerous localities in Switzerland, mainly alpine	2n = 54	Chrysanthemum montanum Allioni (provisional nomenclature)
Favarger & Villard	1966	Several Swiss localities	2n = 72	Chrysanthemum heterophyllum Willd.
Favarger & Villard	1966	Several localities in the maritime Alps and N. Italy	2n = 90	Chrysanthemum leucanthemum L subsp. glaucophyllum. Briquet & Cavillier
Favarger & Villard	1966	Several subalpine localities from the Basses Pyrenees	2n = 108	Chrysanthemum maximum Ramond

APPENDIX III

DETAILS OF THE VARIOUS CYTOLOGICAL TECHNIQUES USED IN THE PRESENT WORK

APPENDIX III

Cytological Techniques

Chromosome analyses were carried out using either actively growing root tips or stamens. Root tips were taken from pot plants and buds from both pot plants and plants grown in order beds. The sources of material and numbers of plants counted are given in Appendix I.

Mitotic material Actively growing roots* were removed and placed in a saturated aqueous solution of α-bromonapthalene for 3 hours, O'Mara (1948), Dowrick (1952). This pretreatment was found to be more suitable than either paradichloro-benzene, hydroxy-quinoline or colchicine. It is essential to make up a fresh solution of α-bromonopthalene each time since stored solutions quickly lose their spindle-inhibiting properties. Fixation was carried out using 3:1, alcohol-acetic and the material stored at -10°C until required for examination. The staining method used was a combined Feulgen-acetocarmine one, carried out by first hydrolysing the roots in 1N Hcl at 60°C for 15 mins.**

---

* Actively growing roots are recognisable by the translucent appearance of the tip as opposed to the whitish opaque appearance in inactive root apical meristems.

** Sharma and Sharma (1965) commenting on Dowrick's (1952) published hydrolysis time of 15 minutes for Chrysanthemum root tips, state that the apparently excessive time required for this hydrolysis is to facilitate chromosome spreading rather than solely for the affect on the staining intensity of the chromosomes. Personal tests have shown that a 15-minute hydrolysis time, whilst not being ultra critical generally gives the highest staining intensity with a wide range of Chrysanthemum leucanthemum L. materials.

and then staining in decolorised Schiffs reagent for 2 hours, finally macerating and squashing the root tip in a drop of aceto-carmine. Slides were made permanent using the dry ice method of Conger and Fairchild (1953).

Meiotic material Buds of approximately 5 m.m. in diameter were fixed in a mixture of 2 parts glacial acetic, 2 parts chloroform to 3 parts alcohol, a fixative recommended by Catcheside (1945) for Composites. The buds were split into four to facilitate the penetration of the fixative. It was found that buds collected earlier than 9.00 a.m. and later than 11.30 a.m. were of little cytological value since diakinesis and metaphase were passed through at this time of day. Material was stored in the fixative at -10°C until required for examination. For staining, the florets were scraped out of the capitulum, hydrolysed in 1N Hcl for 15 minutes at 60°C* and stained in decolorised Schiff's reagent for 2 hours. The florets were then washed in SO_2 water. The anthers were removed from the florets under a dissecting microscope and the contents squeezed out into a drop of aceto-carmine. At this point, examination of P.M.C's under the dissecting microscope showed whether or not the material was at the correct stage or not. If so, than the anther debris was removed, the slide squashed and made permanent using the dry ice method.

---

* This hydrolysis time differs from that of Dowrick's (1952) time who says that 5 minutes is the maximum required for meiotic material in Chrysanthemum. Personal tests showed that a time of less than 10 minutes resulted in gross understaining of the material.

Pollen Grain Analysis

Pollen grains were stained in acetocarmine by mashing up florets in the stain, warming gently and removing the anther debris. A cover-slip was placed on the slide and the specimen examined. Grains were only regarded as fertile if their nuclei stained up. Care was taken to scan across the whole slide evenly since there was a predisposition for sterile, empty grains to float towards the edges of the coverslip.

Another technical point to be observed was the prevention of drying out of the 'wet mount' since this resulted in a lowering of the coverslip and an apparent increase in pollen grain diameter due to squashing.

APPENDIX IV

COMMENTS ON THE TECHNIQUE OF CHROMOSOMAL PHOTOMICROGRAPHY

APPENDIX IV

Photomicrographic Techniques

Most modern photomicrography is carried out using 35 m.m. roll film and this gives rise to problems not normally encountered whilst using film of a larger format. The fact that successful chromosome staining produces an intense colour difference between chromatin and its surrounds has engendered the policy amongst chromosome cytologists of using photographic materials and methods which will not only maintain but enhance the contrast in the subject being photographed. Chromosomes have a rather indistinct edge to them (this varies with the organism and pretreatment used) when critically viewed under a good optical system. When photographed on 35 m.m. film using 'Contrasty' processing, chromosomes lose their indistinct edge and look like sticks which may result in a 'pretty' picture, but is not an accurate representation of the observed appearance down the microscope. The problem of using 'contrasty' materials does not arise when using plates since the number of silver grains per chromosome is so much higher and hence detail at the edge of chromosomes is not lost so easily.

To obtain microphotographs of the necessary medium contrast to avoid 'sticks', Kodak recommend Pan-X developed in D-76. This gives good results but not as good as using microfile, a film of very small grain, low speed and high contrast developed in microdol-X. The high

contrast of the film is compensated by the low contrast of the developer, resulting in negatives of very fine grain and medium contrast. This combination is not recommended by Kodak probably because of the critical processing and longer exposure required. In figs. 59 to 62 are much enlarged prints of the same chromosome photographed using different materials and processing.

Two other emulsions which have recently come to my notice and seem to have the edge over microfile are the Scientia 62 and 56 products of Agfa-Gevaert. These are designed specifically for photomicroscopy and have certain desireable characteristics including an upper colour sensitivity range of 620 and 560 mu respectively, low light scattering properties, a very high resolving power and a brightness of image which cannot be attributed solely to contrast.

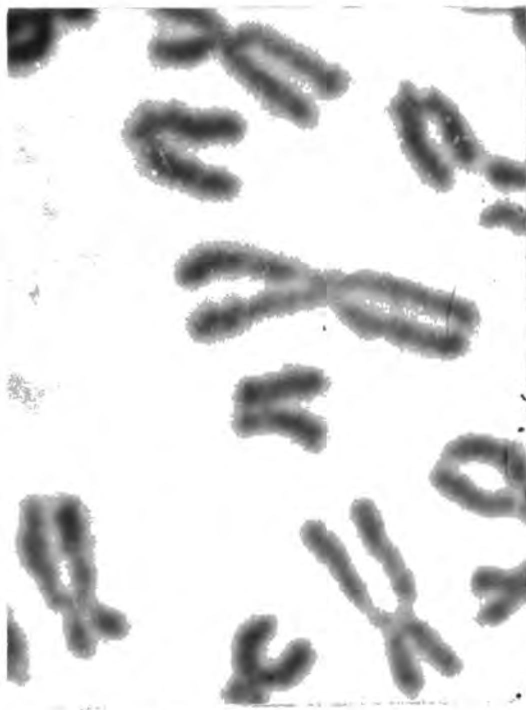


Fig. 59. 35 m.m. Pan-X developed in D-76, printed on Grade 4 Bromide

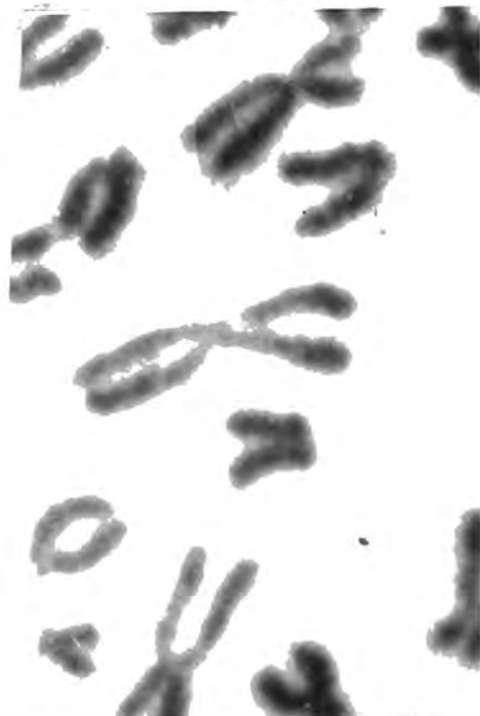


Fig. 60. 35 m.m. Pan-X, developed in Microdol-X printed on Grade 3 Bromide

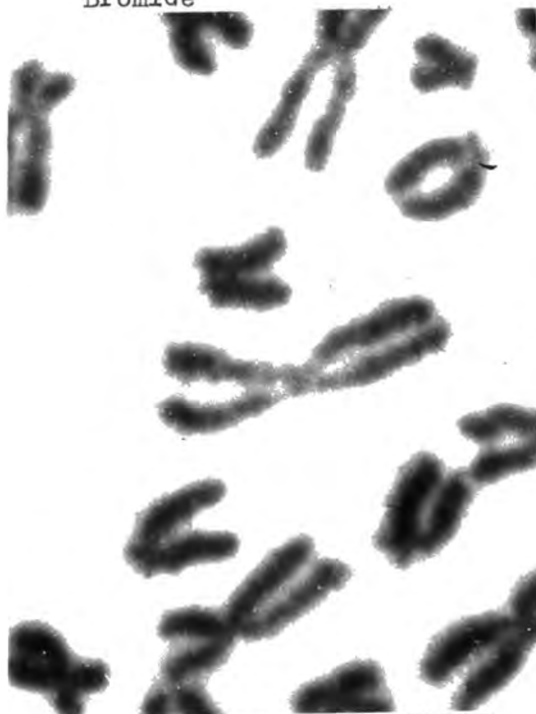


Fig. 61. 35 m.m. Microfile, developed in Microdol-X, printed on Grade 3 Bromide. Results most closely resemble those of the plate in terms of detail.

Fig. 62. Plate 5" x 4" Orto type 3. Developed in Universal, printed on Grade 3 Bromide.

APPENDIX V

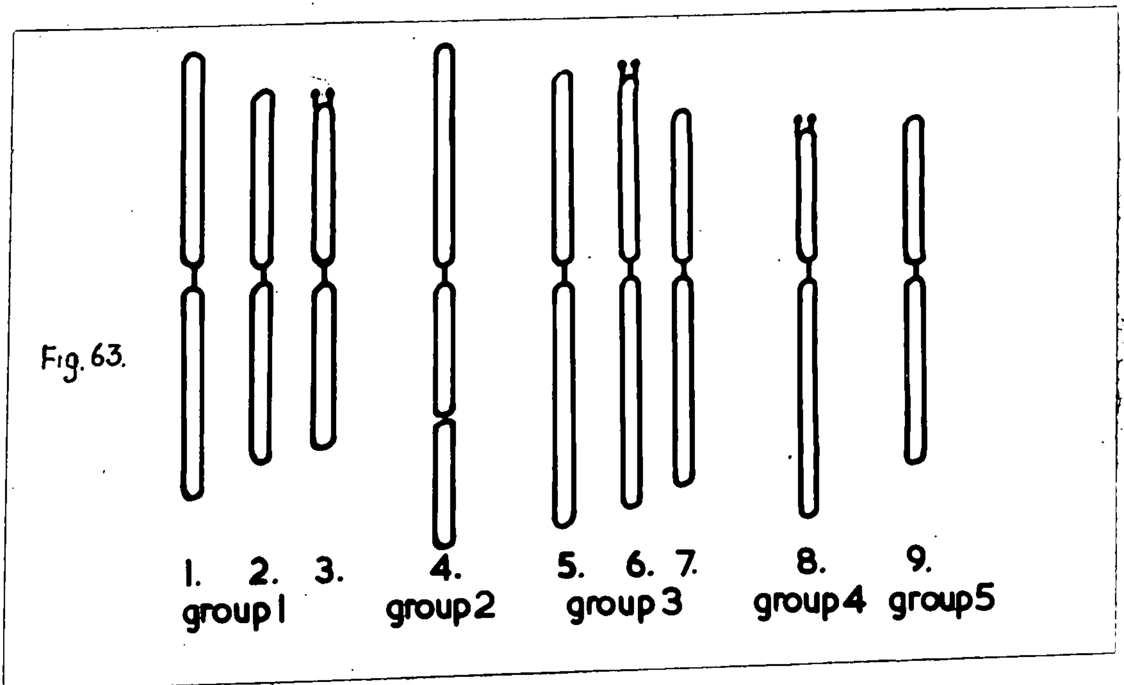
KARYOTYPE ANALYSES IN CHRYSANTHEMUM LEUCANTHEMUM L. SENSU LATO

APPENDIX V

Chromosome marker statistics indicate that chromosomes can vary by up to 6% relative to the rest of the complement. Consequently examination of few cells can give an erroneous impression of chromosome morphology. A 6% variation means that only chromosomes with widely differing morphology can consistently be distinguished. In the following karyotypes the chromosomes have been paired by convention and also to assist in establishing the groups. It is emphasised that paired chromosomes do not mean that the author necessarily considers them as being homologous but rather that they show the best morphological resemblance in the cell concerned.

Diploid karyotypes

Karyotypes of a range of British diploid plants from 5 different populations are given. Chromosome variation appears to fit into a pattern given in Fig. 63 page 152 .



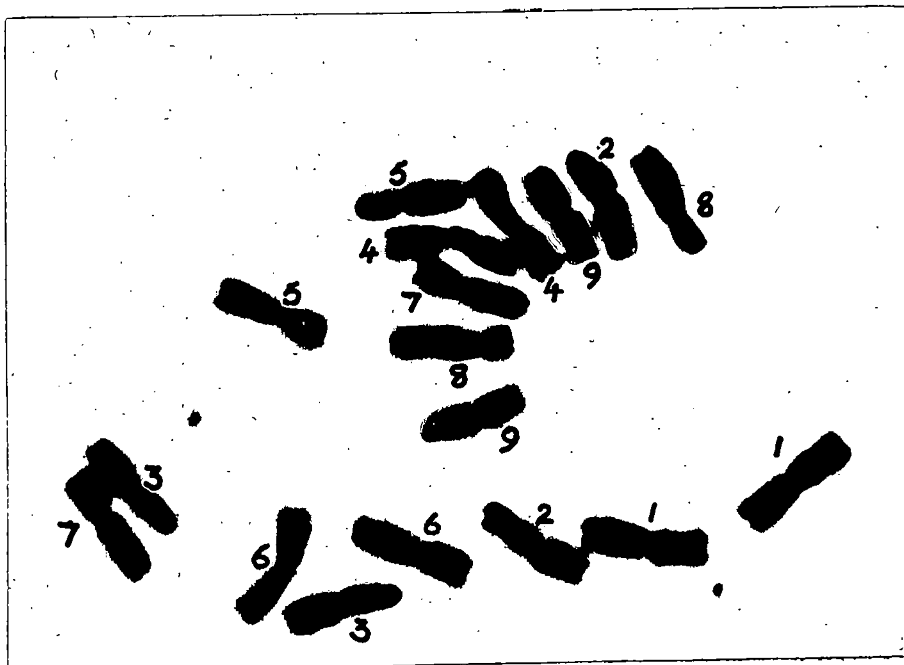


Fig. 64. The chromosomes are numbered according to the idiogram given in Fig. 63 In this cell chromosomes 2 and 3 and 5 and 6 are indistinguishable.

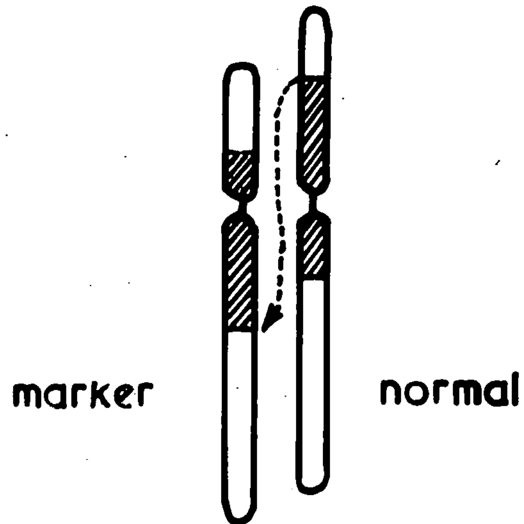


FIG.65. SUGGESTED INVERTED REGION ON GROUP 4 CHROMOSOME

GROUP 1

GROUP 2

GROUP 3

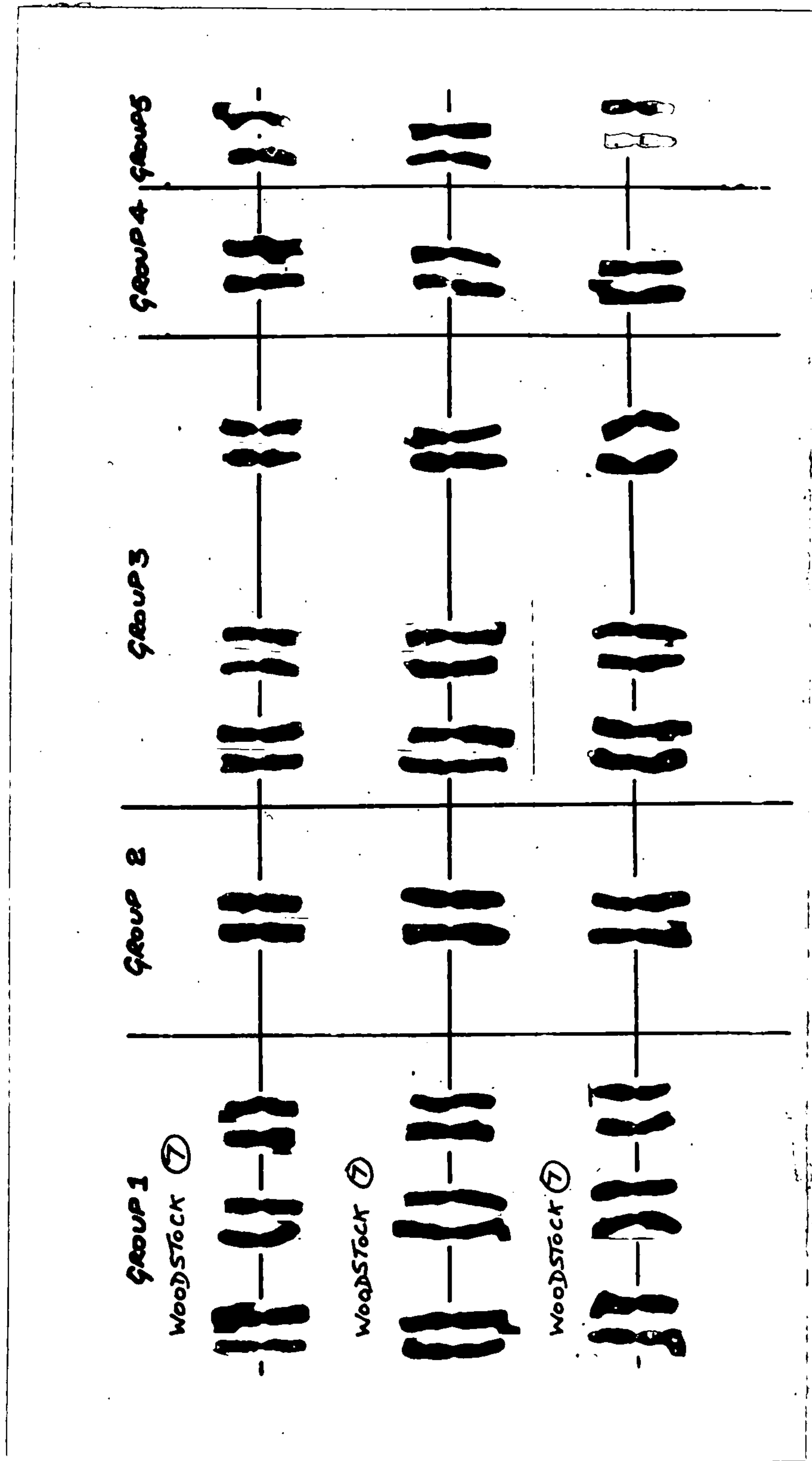
GROUP 4

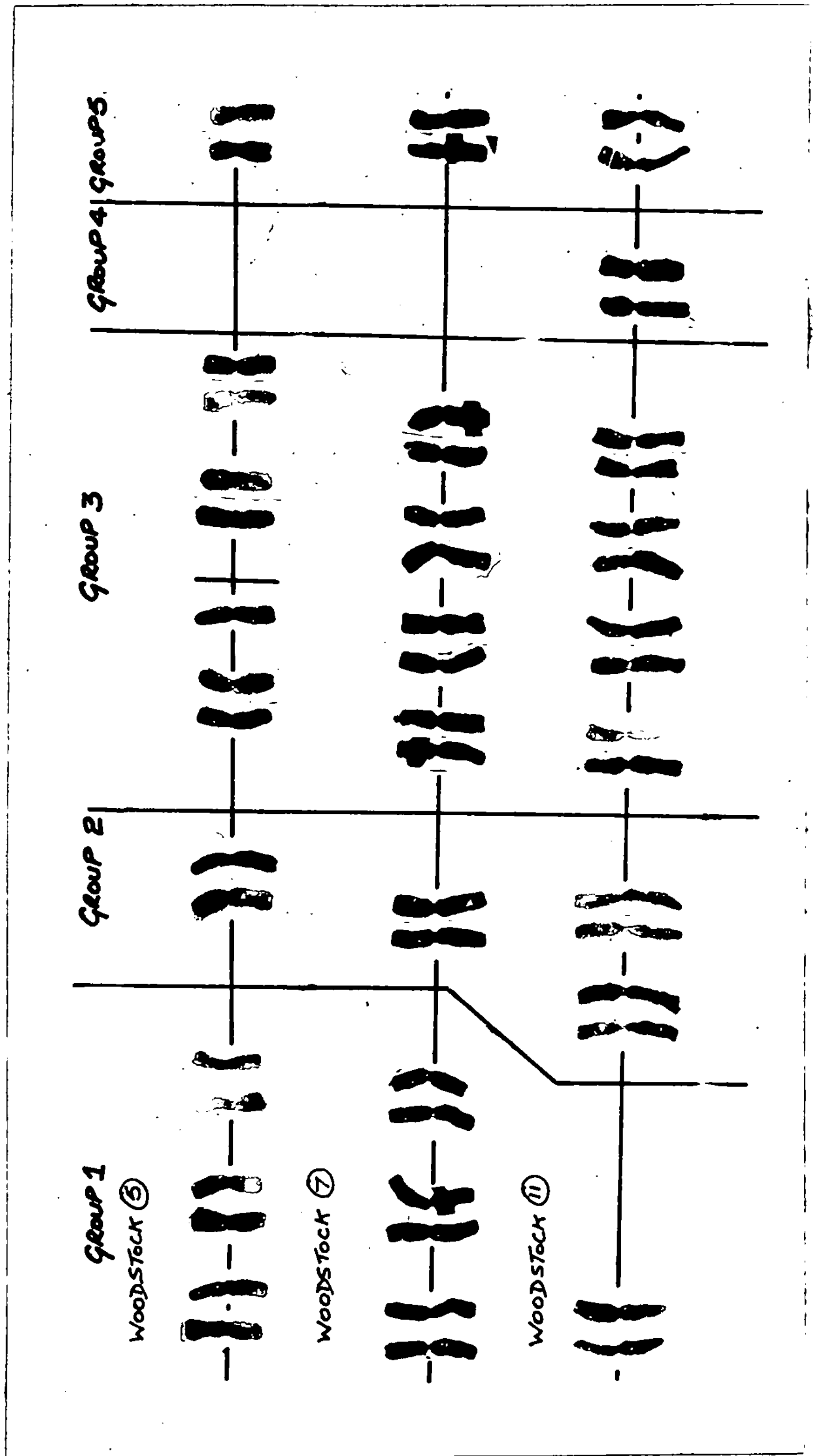
GROUP 5

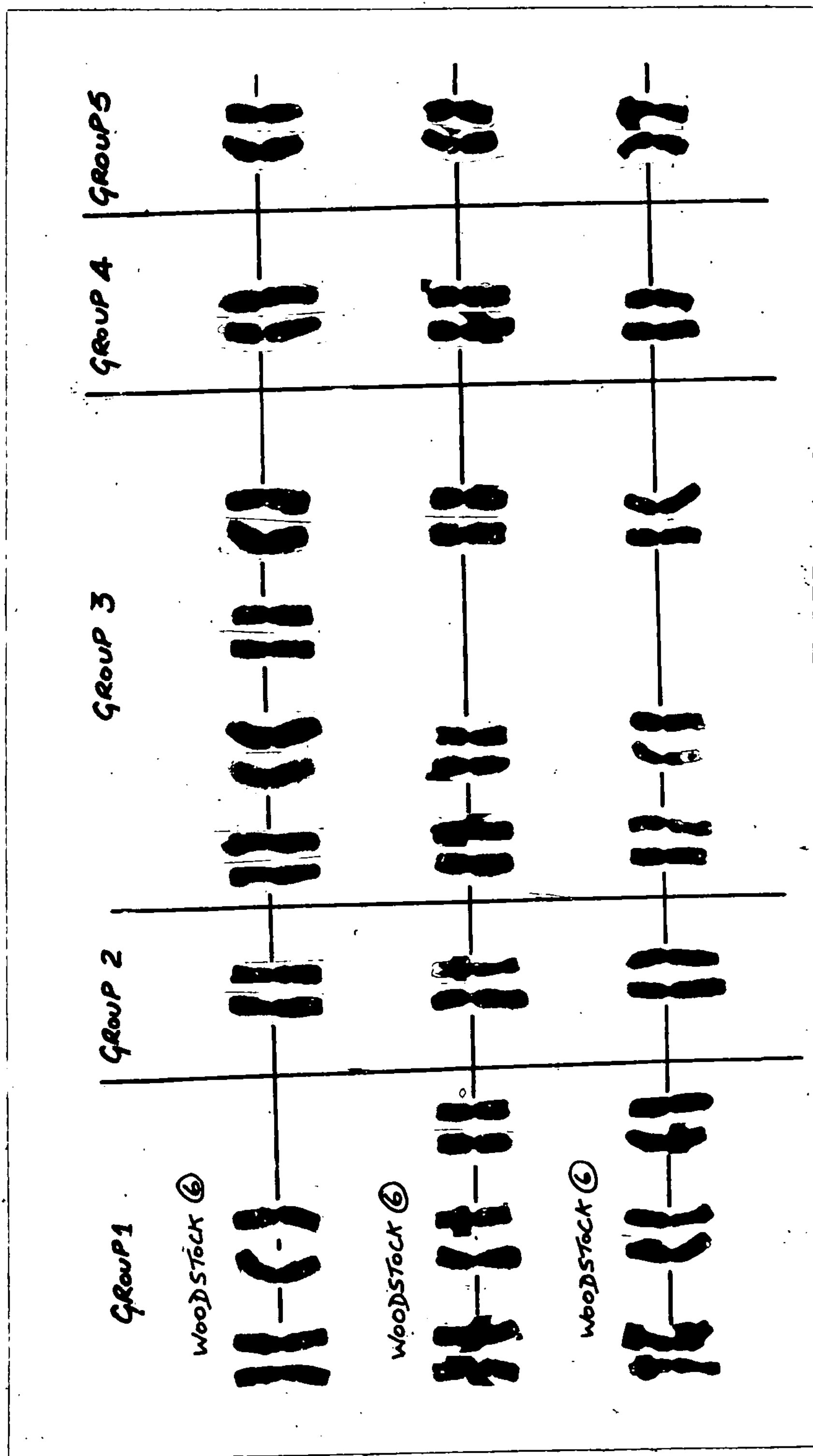
LITTLE HAVEN (3)

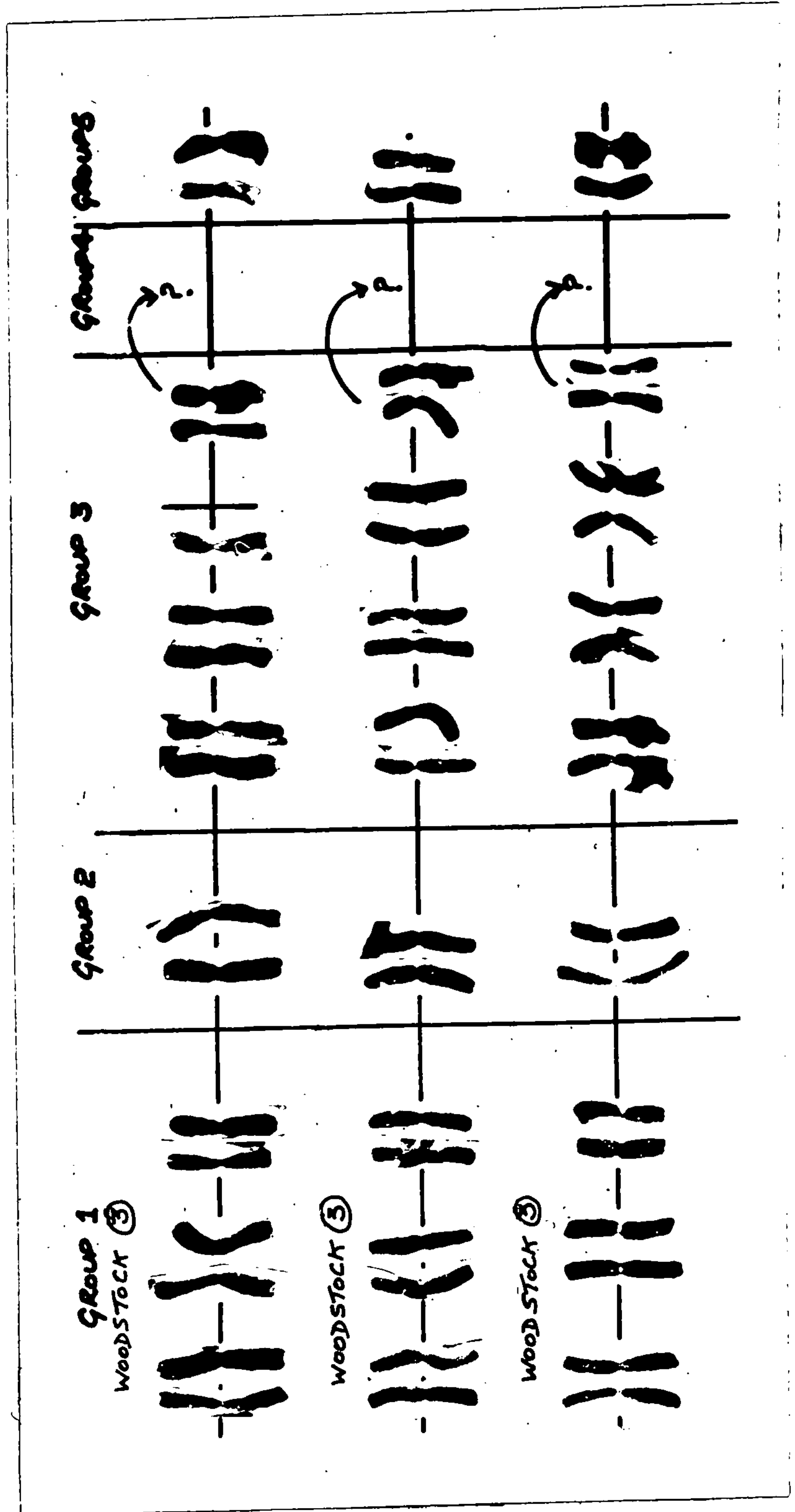
LITTLE HAVEN (2)

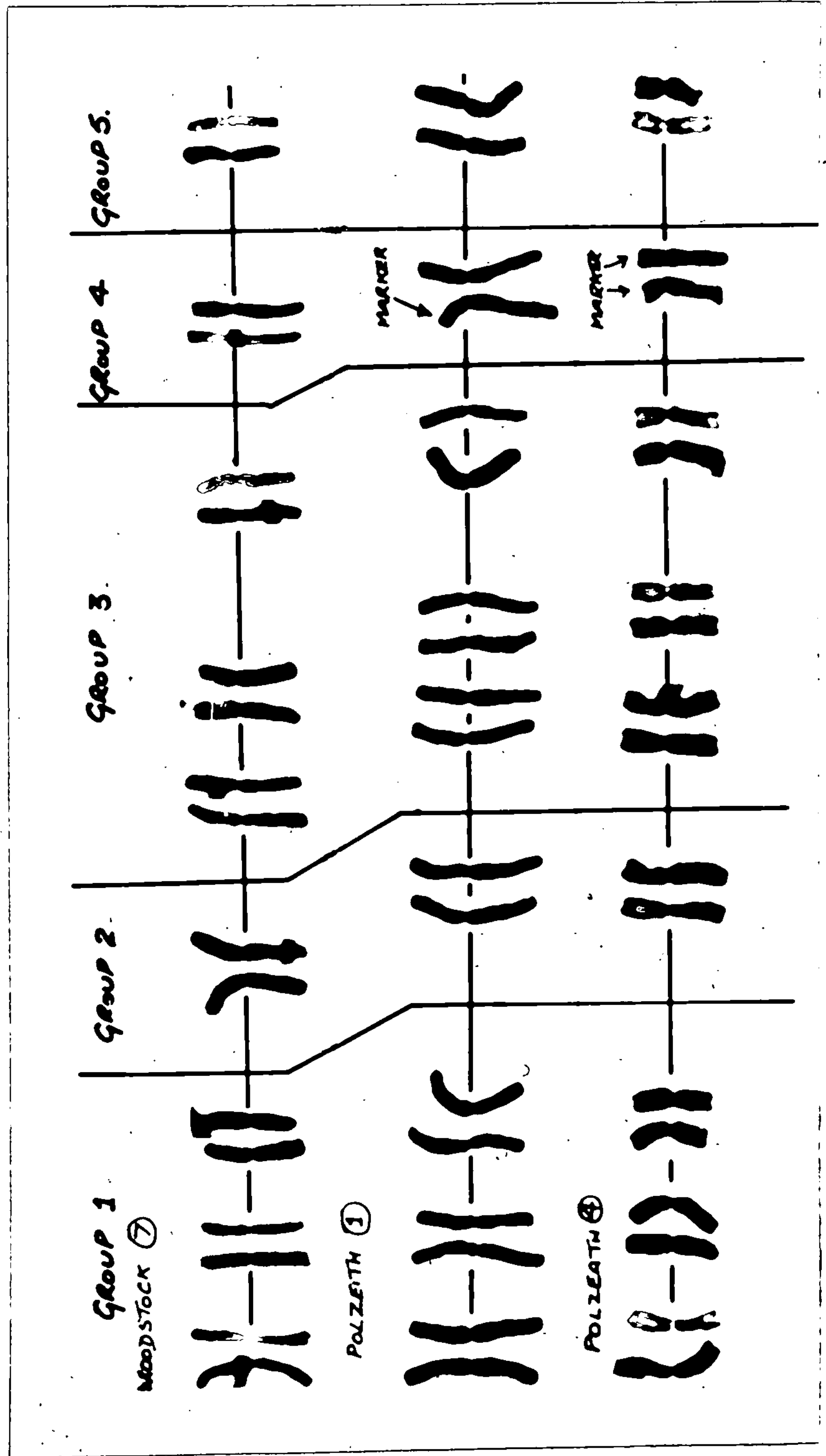
CARNEDD (2)

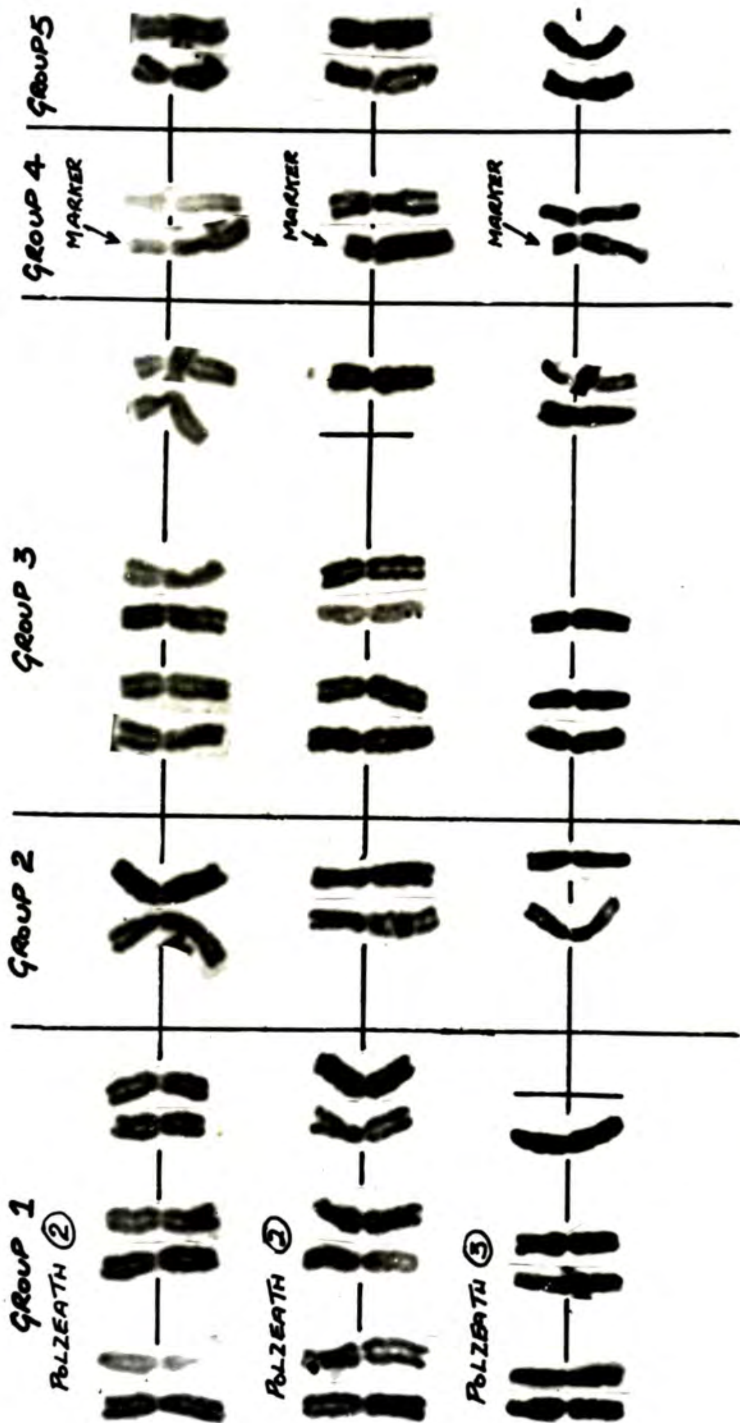

GROUP 1
WOODSTOCK 7
WOODSTOCK 7
WOODSTOCK 7
GROUP 2
GROUP 3
GROUP 4
GROUP 5

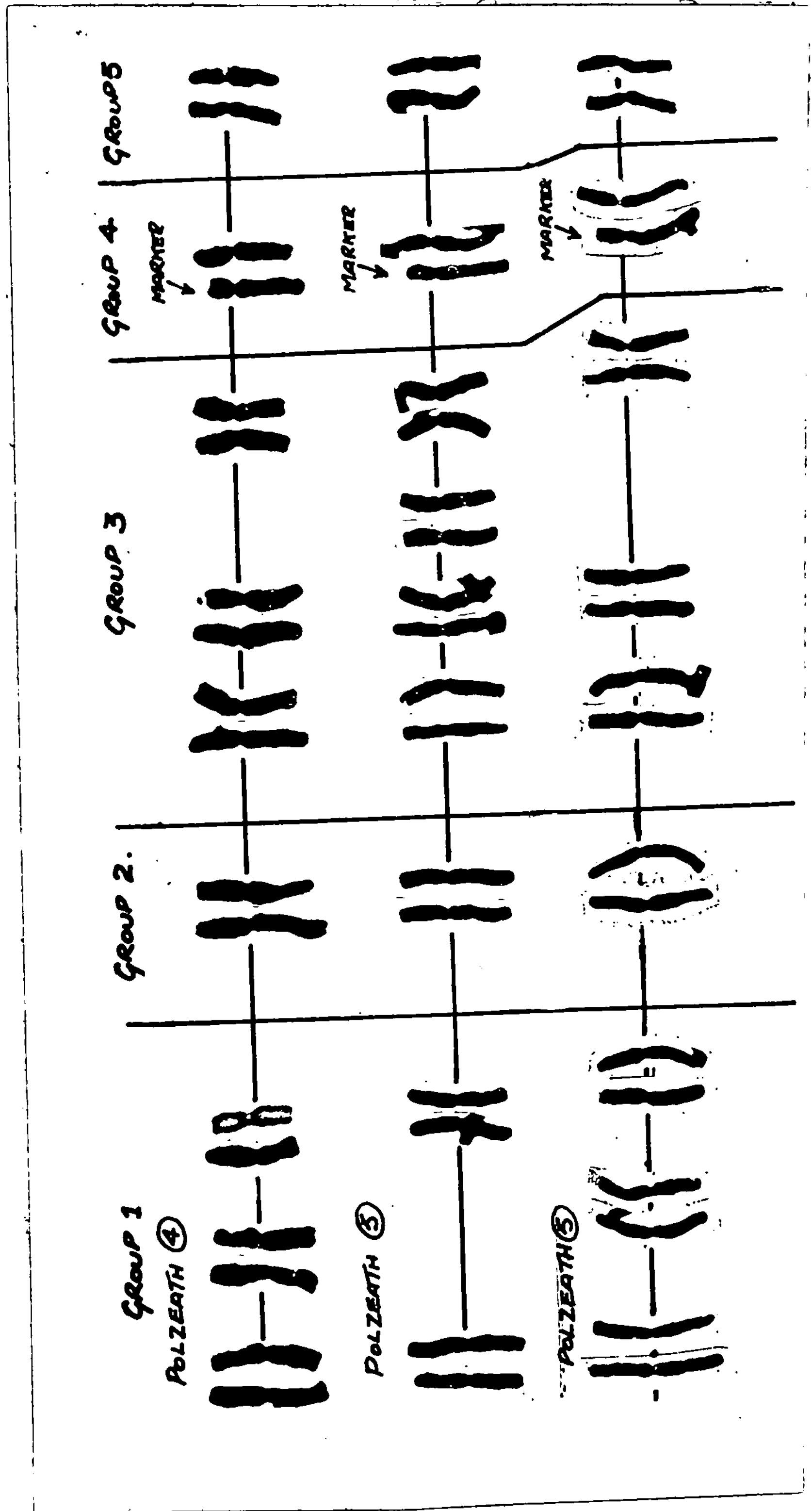

GROUP 1
GROUP 2
GROUP 3
GROUP 4
GROUP 5
WOODSTOCK (5)
WOODSTOCK (7)
WOODSTOCK (11)

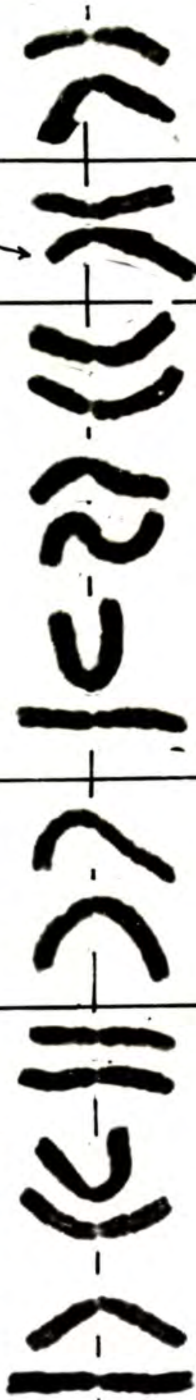

GROUP 1
WOODSTOCK ⑥
WOODSTOCK ⑥
WOODSTOCK ⑥
GROUP 2
GROUP 3
GROUP 4
GROUP 5


GROUP 1
GROUP 2
GROUP 3
GROUP 4
GROUP 5
WOODSTOCK ③
WOODSTOCK ③
WOODSTOCK ③
a.
a.
a.


GROUP 1
WOODSTOCK ⑦
POLZEATH ①
POLZEATH ④
GROUP 2
GROUP 3
GROUP 4
GROUP 5.
MARKER
MARKER


GROUP 1
GROUP 2
GROUP 3
GROUP 4
GROUP 5
POLZEATH ②
POLZEATH ①
POLZEATH ③
MARKER
MARKER
MARKER


GROUP 1
POLZEATH (4)
POLZEATH (5)
POLZEATH (5)
GROUP 2.
GROUP 3
GROUP 4
MARKER
MARKER
MARKER
GROUP5

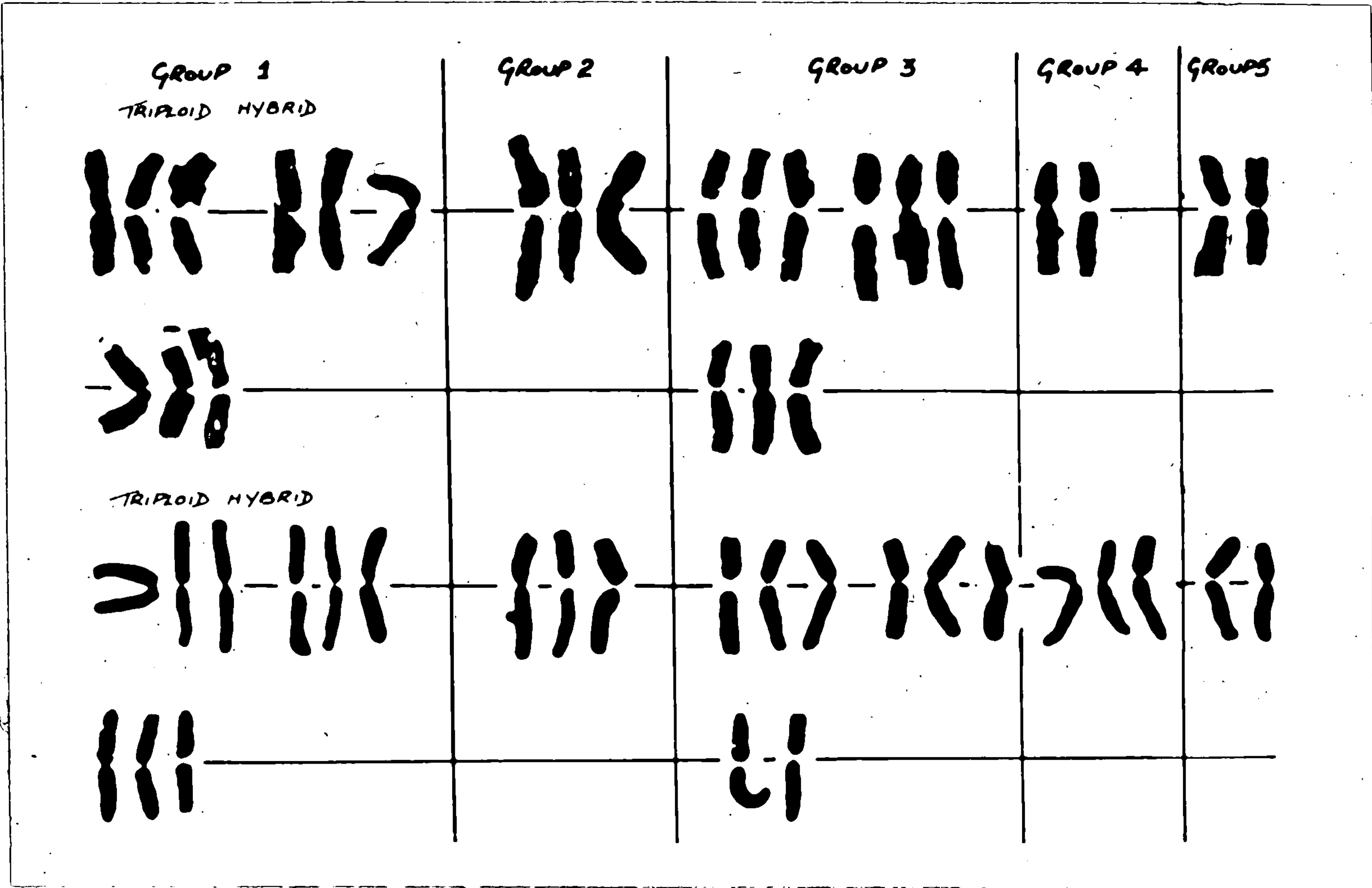

GROUP 1
MONKHAVEN (1)
GROUP 2.
GROUP 3.
GROUP 4
MARKER
GROUP 5

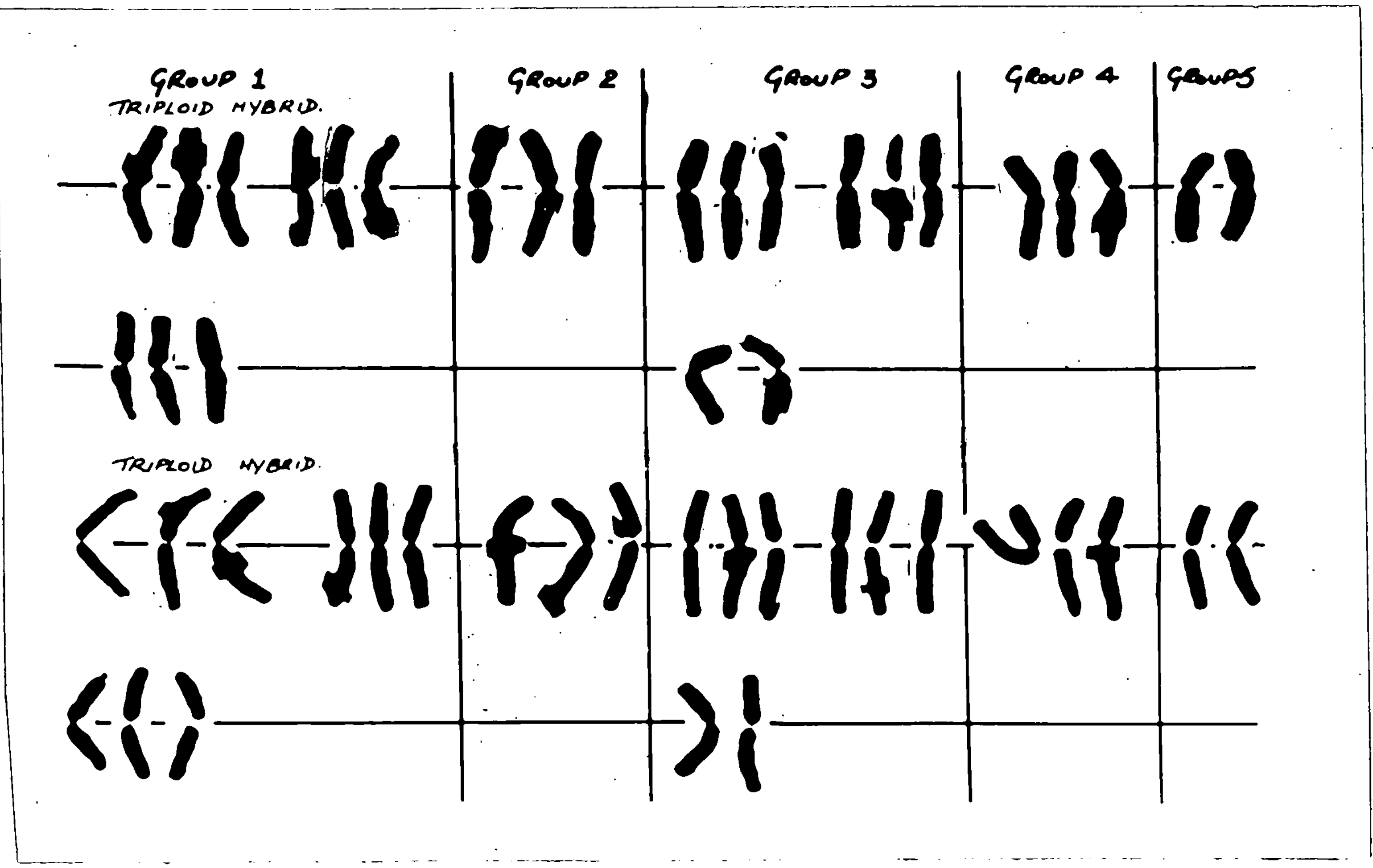
POLZEATH (1)
MARKER

POLZEATH (1)
MARKER

Triploid karyotypes

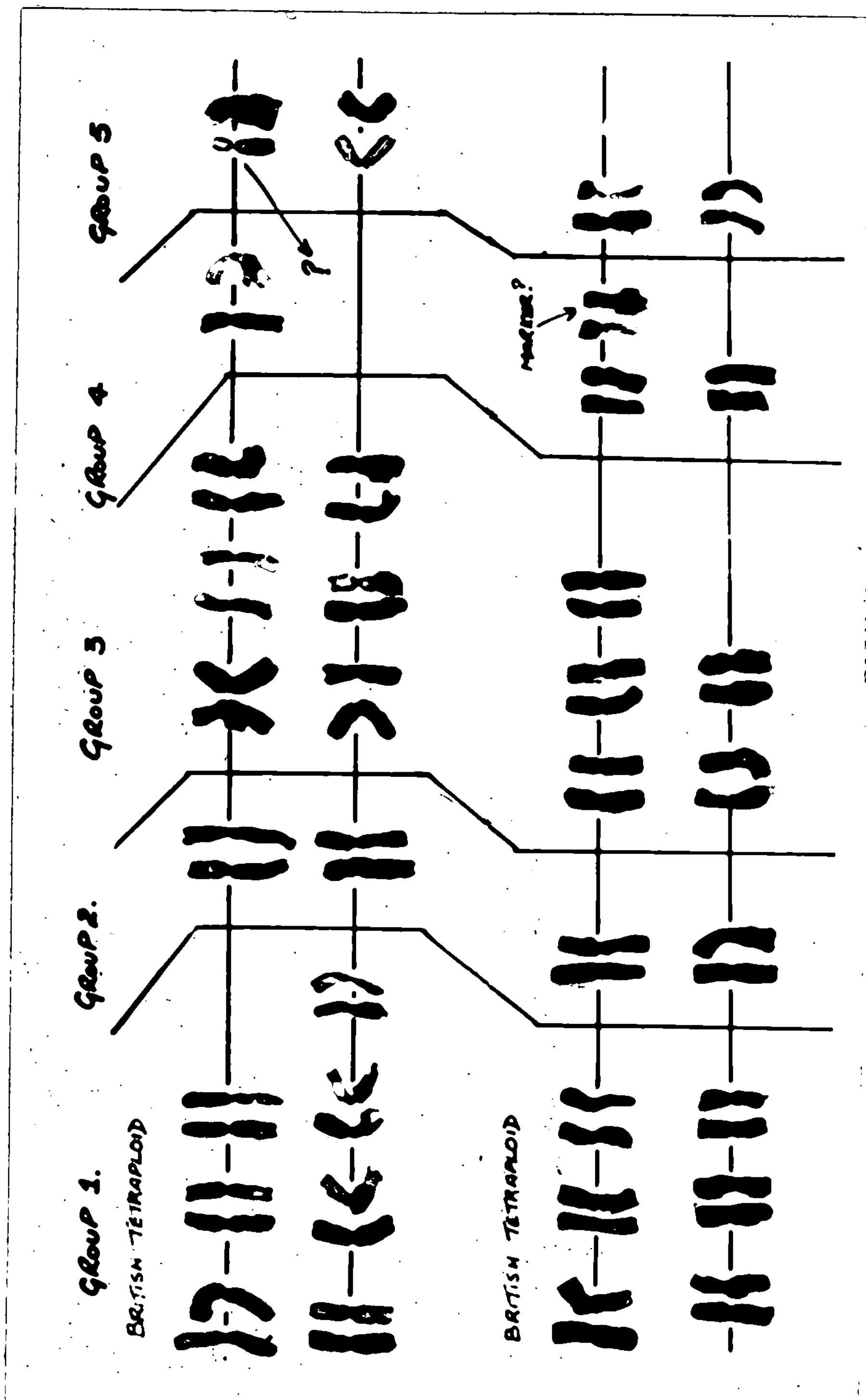
The four cells analysed are from a 25 chromosome triploid. Meiotic evidence indicated that the two missing chromosomes were non-homologous and this assumption has been made whilst pairing the chromosomes. It can be seen that there are indications of trisomy on the basis of the way the chromosomes have been arranged. Bearing in mind the possible 6% variation in size and shape of any one chromosome, bivalents and univalents could probably be derived. However, the chromosomes do seem to fall more easily into three's by intention or otherwise.

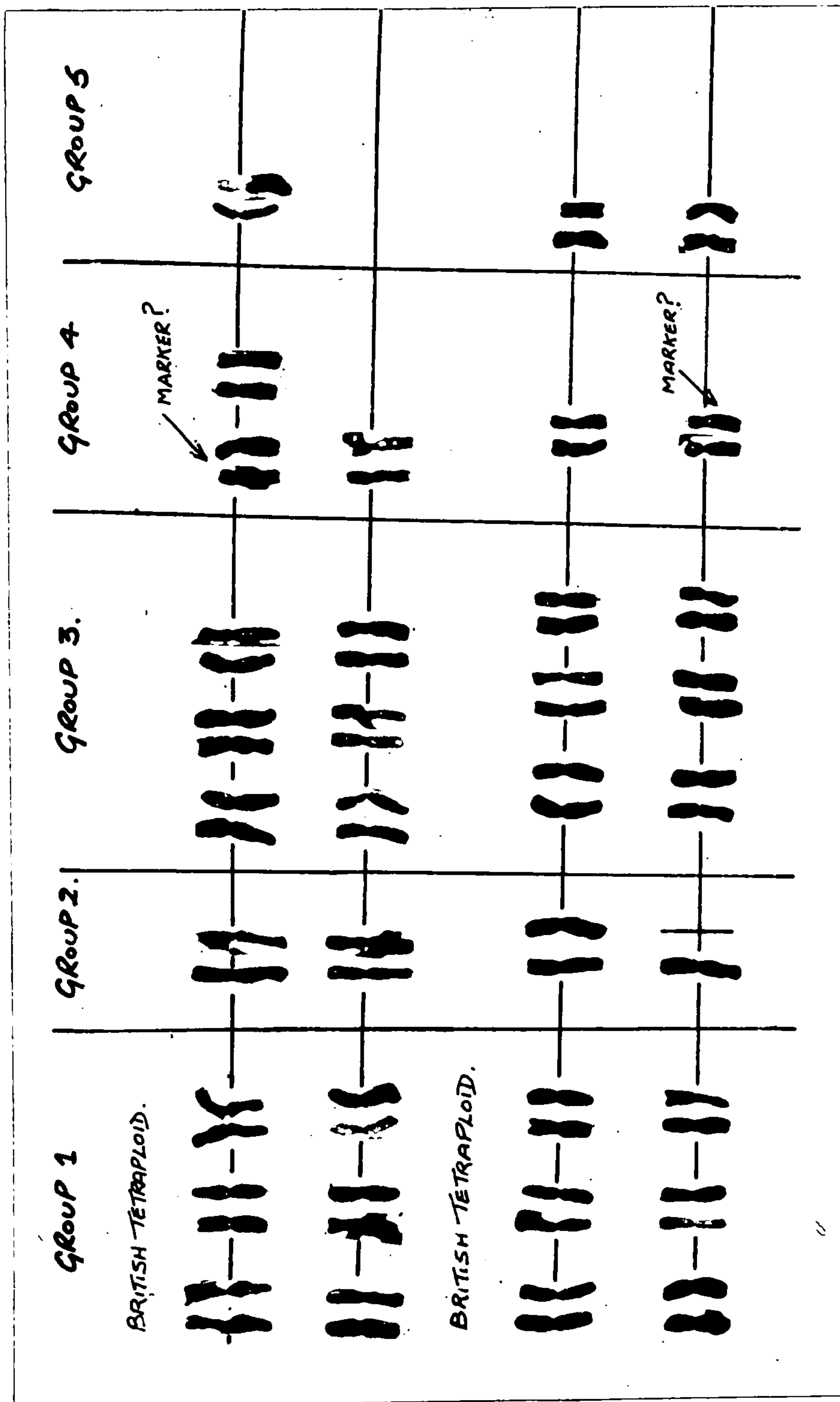

GROUP 1
TRIPLOID HYBRID
GROUP 2
GROUP 3
GROUP 4
GROUP 5
TRIPLOID HYBRID

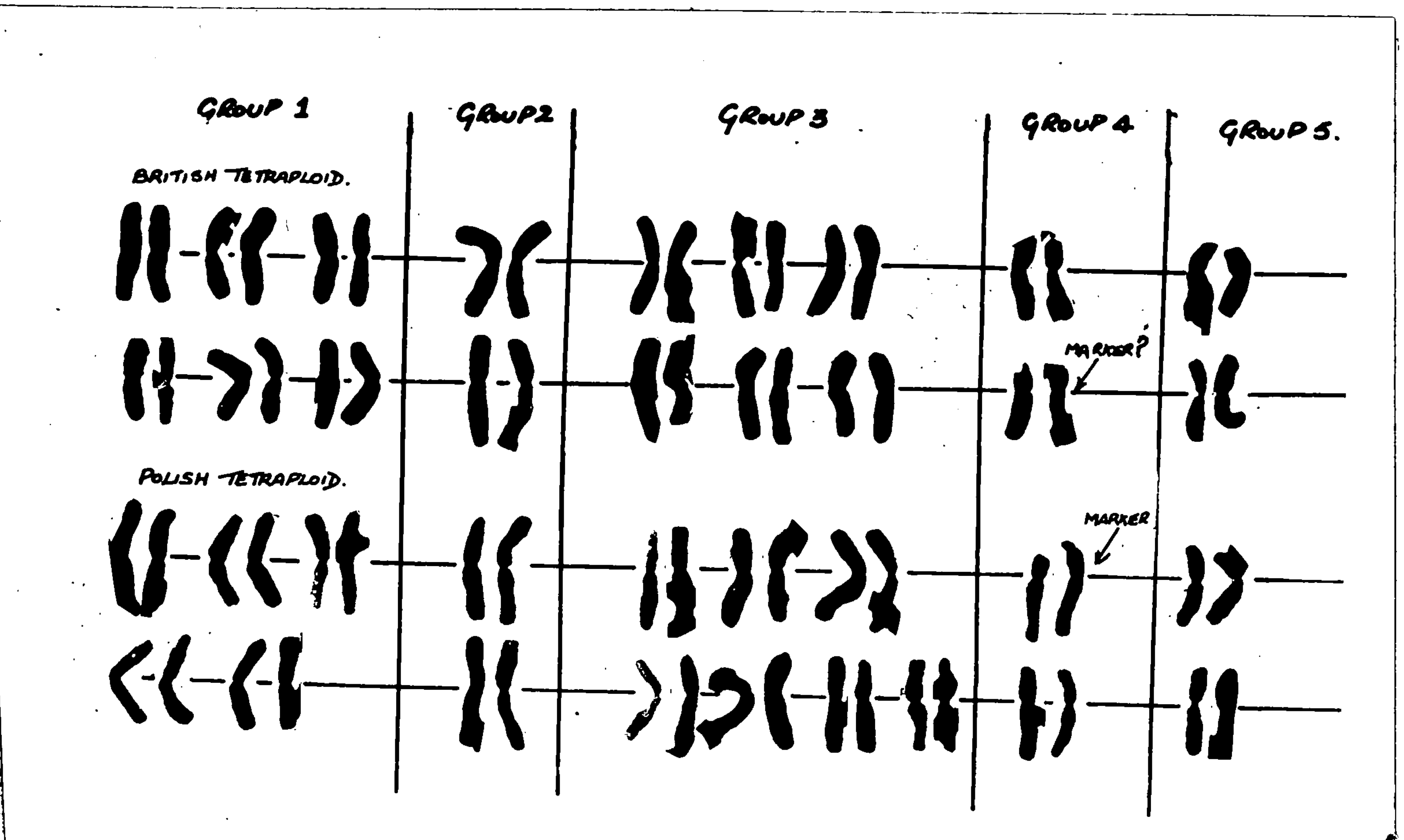

GROUP 1
TRIPLOID HYBRID.
GROUP 2
GROUP 3
GROUP 4
GROUP 5
TRIPLOID HYBRID.

Tetraploid karyotypes

Karyotypes of British and Polish tetraploids are given. One pair of group four chromosomes resembles the Cornish diploid marker chromosome and has been noted in all tetraploid populations examined. Disomy is indicated by some chromosomes and tetrasomy, viz. group 2, by others.


GROUP 1.
BRITISH TETRAPLOID
BRITISH TETRAPLOID
GROUP 2.
GROUP 3
GROUP 4
MARKER?
GROUP 5
?


GROUP 1
GROUP 2.
GROUP 3.
GROUP 4
GROUP 5
BRITISH TETRAPLOID.
BRITISH TETRAPLOID.
MARKER?
MARKER?


GROUP 1
GROUP 2
GROUP 3
GROUP 4
GROUP 5.
BRITISH TETRAPLOID.
POLISH TETRAPLOID.
MARKER?
MARKER

APPENDIX VI

PRINTOUT OF PROGRAM AND RESULTS OF THE AUTOTETRAPLOID MEIOTIC PAIRING ROUTINE

```
chiasma frequency in autotetraploids version 1b jan65;
begin integer array end[1:2,1:9,1:4];
        integer int1,int2,int3,n,y,z,x,w,q,one,two,posn,set,chrom,
                p,keyword,univ,rod,rod2,rod3,mult,mult2,rod4,
                mult3,mult4,rodbiv,triv,rodquad,ringquad,ringbiv;
        switch ss:=label1,label2,label3;
        procedure randomise(int1,int2);
integer int1,int2;
begin integer k,one,storac,storar,i1,i2;
switch sw:=iterate,exit,repeat;
k:=455470314;one:=1;
repeat:     elliott(3,0,keyword,0,5,2,k);
              elliott(2,0,storac,0,5,7,0);
            elliott(2,0,storar,0,0,0,0);
iterate: elliott(3,0,storar,0,5,0,38);
            elliott(3,0,storac,0,0,0,0);
            elliott(5,4,7,0,1,6,keyword);
            elliott(5,0,7,0,5,7,0);
            elliott(1,0,keyword,0,2,0,storac);
          if storac=0 then goto exit;
         elliott(3,0,storac,0,0,0,0);
            elliott(0,4,keyword,0,5,2,one);
          elliott(2,0,storac,0,5,7,0);
          elliott(2,0,storar,0,0,0,0);
          goto iterate;
exit: i1:=511; i2:=1536;
         elliott(3,0,keyword,0,2,3,i1);
         elliott(0,3,i2,0,5,1,9);
         elliott(2,0,i2,0,0,0,0);
           int1:=i1/7; int2:=i2+1;
           if int1=0 or int1=73 then goto repeat;
end;
      univ:=rodbiv:=triv:=rodquad:=ringquad:=ringbiv:=0;
        keyword:=262143;
        elliott(0,6,0,0,7,0,0);
        elliott(2,3,keyword,0,0,0,0);

                    for n:=1 step 1 until 30 do

                begin for w:=1 step 1 until 72 do

                    begin posn:=entier((w+35)/36);
                              if w>36 then set:=entier(((w-36)+3)/4)
                             else set:=entier((w+3)/4);
                             if w>36 then chrom:=((w-36)+4)-(set*4) else
                                     chrom:=(w+4)-(set*4);
                      end[posn,set,chrom]:=0;   end;
                    for z:=1 step 1 until 27 do
                     begin    label1:   randomise(int1,int2);one:=two:=0;
                      posn:=entier((int1+35)/36);
                     if int1>36 then set:=Ientier(((int1-36)+3)/4 i
                    else set:= entier((int1+3)/4);
                     if int1>36 then
                      chrom:=((int1-36)+4)-(set*4) else
                      chrom:=(int1+4)-(set*4);
                    for q:=1 step 1until 4 do
                    begin if end[posn,set,q]=1 then
                     one:=one+1 else if  end[posn,set,q]=2 then
                    two:=two+1;
                     end;
                    if two>0 then goto label1;
                    if one>0 then begin
                     if end[posn,set,chrom]=0 then
                   end[posn,set,chrom]:=2 else goto label1;
              for q:=1step 1 until4 do
                begin if end[posn,set,q]=0 then end[posn,set,q]:=2; end;


                     if end[posn,set,chrom]=0 then
                   end[posn,set,chrom]:=2 else goto label1;
              for q:=1step 1 until4 do
                begin if end[posn,set,q]=0 then end[posn,set,q]:=2; end; end
(                   else begin end[posn,set,chrom]:=1;
                     if int2=chrom then begin
              label3:      randomise(int1,int2);
                    if int2=chrom then goto label3 else
                     end[posn,set,int2]:=1; end else end[posn,set,int2]:=1; end;
                    end; p:=1;for w:=1 step 1 until 72 do

                     begin posn:=entier((w+35)/36);
                              if w>36 then set:=entier(((w-36)+3)/4)
                             else set:=entier((w+3)/4);
                             if w>36 then chrom:=((w-36)+4)-(set*4) else
                                       chrom:=(w+4)-(set*4);
             print end[posn,set,chrom];   end;
                       for q :=1 step 1 until 9 do
                        begin  rod:=rod2:=rod3:=mult:=mult2:=rod4:=
                             mult3:=mult4:=0; for w:=1 step 1 until 4 do
                      begin  if end[p,q,w]=0 and end[p*2,q,w]=0
                        then univ:=univ+1    else
                        if end[p,q,w]=0 and end[p*2,q,w]=1 then
                              rod:=rod+1   else
                      if end[p,q,w]=0 and end[p*2,q,w]=2 then
                      rod2:=rod2+1    else
                     if end[p,q,w]=1 and end[p*2,q,w]=0 then
                        rod3:=rod3+1   else
  (                if end[p,q,w]=1 and end[p*2,q,w]=1 then
     )                         mult:=mult+1  else
                    if end[p,q,w]=1 and end[p*2,q,w]=2 then
                       mult2:=mult2+1  else
                    if end[p,q,w]=2 and end[p*2,q,w]=0 then
                         rod4:=rod4+1  else
                  if end[p,q,w]=2 and end[p*2,q,w]=1 then
                      mult3:=mult3+1  else
                    if end[p,q,w]=2 and end[p*2,q,w]=2 then
                       mult4:=mult4+1 ;
                       end;
                       print univ,sameline,rod,rod2,rod3,mult,mult2,rod4,mult3,mult4;   0
              if  rod=2 then rodbiv:=rodbiv+1;
                    if rod2=2 then rodbiv:=rodbiv+1;
                    if rod3=2 then rodbiv:=rodbiv+1;
                   if rod4=2 then rodbiv:=rodbiv+1;
                    if rod=1 and mult=1 and rod3=1 then triv:=
                     triv+1;
                    if rod=1 and mult=1 and mult2=1 and rod2=1
                    then rodquad:=rodquad+1;
                   if rod3=1 and mult=1 and mult3=1 and rod4=1
                     then rodquad:=rodquad+1;
                  if mult=1 and mult3=1 and mult4=1 and  mult2=1
                   then ringquad:=ringquad+1;
                  if mult=2 then ringbiv:=ringbiv+1;
                  if mult2=2 then ringbiv:=ringbiv+1;
                  if mult3=2 then ringbiv:=ringbiv+1;
                  if mult4=2 then ringbiv:=ringbiv+1;  end;
print £n?,sameline,n,£  ?;
print £ringbiv?,sameline,(ringbiv*2)/(36*n),£  ?;
print £rodbiv?,sameline,(rodbiv*2)/(36*n),£  ?;
print £univ?,sameline,univ/(36*n),£
?;
print £triv?,sameline,(triv*3)/(36*n),£  ?;
print £ringquad?,sameline,(ringquad*4)/(36*n),£  ?;
print £rodquad?,sameline,(rodquad*3)/(36*n),£
?;
end; end; end;
```

VERSION 1.

chiasma frequency in autotetraploids version ½ jan65

Free Store = 5040 to 5226

number 1 univ 6.0000000 ringbiv 4.0000000 rodbiv 8.0000000
triv 6.0000000 ringquad .00000000 rodquad 12.000000
number 2 univ 5.5000000 ringbiv 3.0000000 rodbiv 9.0000000
triv 4.5000000 ringquad 2.0000000 rodquad 12.000000
number 3 univ 5.3333333 ringbiv 4.0000000 rodbiv 9.3333333
triv 4.0000000 ringquad 1.3333333 rodquad 12.000000
number 4 univ 4.2500000 ringbiv 4.5000000 rodbiv 11.500000
triv 3.7500000 ringquad 1.0000000 rodquad 11.000000
number 5 univ 4.0000000 ringbiv 3.6000000 rodbiv 10.800000
triv 4.8000000 ringquad 1.6000000 rodquad 11.200000
number 6 univ 4.0000000 ringbiv 3.3333333 rodbiv 10.333333
triv 5.0000000 ringquad 2.0000000 rodquad 11.333333
number 7 univ 4.0000000 ringbiv 3.4285714 rodbiv 10.000000
triv 4.2857143 ringquad 2.2857143 rodquad 12.000000
number 8 univ 3.8750000 ringbiv 4.0000000 rodbiv 10.500000
triv 4.1250000 ring uad .5000000 rodquad 11.000000
number 9 univ 3.7777778 ringbiv 4.2222222 rodb v 10.222222
triv 4.0000000 ringquad 2.2222 22 rodquad 11.555556
number 10 univ 3.6000000 ringbiv 4.4000000 rodbiv 10.000000
triv 3.6000000 ringquad 2.0000000 rodquad 12.400000
number 11 univ 3.5454545 ringbiv 4.3636364 rodbiv 9.6363636
triv 3.5454545 ringquad 2.1818182 rodquad 12.727273
number 12 univ 3.5833333 ringbiv 4.6666667 rodbiv 9.3333333
triv 3.7500000 ringquad 2.3333333 rodquad 12.333333
number 13 univ 3.3846154 ringbiv 4.6153846 rodbiv 9.5384615
triv 3.6923077 ringquad 2.4615384 rodquad 12.307692
number 14 univ 3.4285714 ringbiv 4.4285714 rodbiv 9.5714285
triv 3.4285714 ringquad 3.1428571 rodquad 12.000000
number 15 univ 3.3333333 ringbiv 4.5333334 rodbiv 9.7333333
triv 3.2000000 ringquad 3.4666667 rodquad 11.733333
number 16 univ 3.3125000 ringbiv 4.8750000 rodbiv 9.3750000
triv 3.1875000 ringquad 3.2500000 rodquad 12.000000
number 17 univ 3.2352941 ringbiv 4.8235294 rodbiv 9.4117647
triv 3.0000000 ringquad 3.5294118 rodquad 12.000000
number 18 univ 3.2777778 ringbiv 4.8888889 rodbiv 9.2222222
triv 2.8333333 ringquad 3.7777778 rodquad 12.000000
number 19 univ 3.4210526 ringbiv 5.3684211 rodbiv 8.8421052
triv 3.0000000 ringquad 3.7894737 rodquad 11.578947
number 18 univ 3.2777778 ringbiv 4.8888889 rodbiv 9.2222222
triv 2.8333333 ringquad 3.7777778 rodquad 12.000000
number 19 univ 3.4210526 ringbiv 5.3684211 rodbiv 8.8421052
triv 3.0000000 ringquad 3.7894737 rodquad 11.578947
number 20 univ 3.4000000 ringbiv 5.4000000 rodbiv 8.8000000
triv 3.0000000 ringquad 4.0000000 rodquad 11.400000
number 21 univ 3.4285714 ringbiv 5.4285714 rodbiv 8.4761905
triv 2.8571429 ringquad 4.1904762 rodquad 11.619048
number 22 univ 3.4545455 ringbiv 5.6363636 rodbiv 8.2727273
triv 3.0000000 ringquad 4.3636364 rodquad 11.272727
number 23 univ 3.3478261 ringbiv 5.8260869 rodbiv 8.1739130
triv 3.0000000 ringquad 4.1739131 rodquad 11.478261
number 24 univ 3.3333333 ringbiv 5.7500000 rodbiv 7.9166667
triv 3.0000000 ringquad 4.3333333 rodquad 11.666667
number 25 univ 3.2400000 ringbiv 5.7600000 rodbiv 7.8400000
triv 3.0000000 ringquad 4.3200000 rodquad 11.840000
number 26 univ 3.1923077 ringbiv 6.0000000 rodbiv 8.0769231
triv 2.8846154 ringquad 4.4615385 rodquad 11.384615
number 27 univ 3.2222222 ringbiv 6.2962963 rodbiv 8.0000000
triv 2.7777778 ringquad 4.4444444 rodquad 11.259259
number 28 univ 3.1785714 ringbiv 6.5000000 rodbiv 8.0714286
triv 2.6785714 ringquad 4.4285714 rodquad 11.142857
number 29 univ 3.2068966 ringbiv 6.4827586 rodbiv 7.9310345
triv 2.7931035 ringquad 4.6896552 rodquad 10.896552
number 30 univ 3.1333333 ringbiv 6.6666667 rodbiv 7.9333333
triv 2.8000000 ringquad 4.5333334 rodquad 10.933333
number 31 univ 3.1612903 ringbiv 6.9032258 rodbiv 7.7419355
triv 2.7096774 ringquad 4.5161290 rodquad 10.967742
number 32 univ 3.1250000 ringbiv 6.8125000 rodbiv 7.6875000
triv 2.6250000 ringquad 4.7500000 rodquad 11.000000
number 33 univ 3.0303030 ringbiv 7.0909090 rodbiv 7.8181818
triv 2.5454545 ringquad 4.6060606 rodquad 10.909091
number 34 univ 3.0000000 ringbiv 7.1764706 rodbiv 7.7058824
triv 2.4705882 ringquad 4.5882353 rodquad 11.058824
number 35 univ 3.0000000 ringbiv 7.2571428 rodbiv 7.6000000
triv 2.4857143 ringquad 4.6857143 rodquad 10.971429
number 36 univ 2.9722222 ringbiv 7.2222222 rodbiv 7.4444444
triv 2.5833333 ringquad 4.7777778 rodquad 11.000000
number 37 univ 2.9189189 ringbiv 7.1891892 rodbiv 7.2972973
triv 2.5945946 ringquad 4.8648649 rodquad 11.135135
number 38 univ 2.8684211 ringbiv 7.1578947 rodbiv 7.1578947
triv 2.6052632 ringquad 4.9473684 rodquad 11.263158
number 39 univ 2.7948718 ringbiv 7.0769231 rodbiv 7.1794872
triv 2.5384616 ringquad 5.1282051 rodquad 11.282051
number 40 univ 2.7500000 ringbiv 7.1000000 rodbiv 7.2000000
triv 2.5500000 ringquad 5.3000000 rodquad 11.100000
triv 2.5945946 ringquad 4.8648649 rodquad 11.135135
number 38 univ 2.8684211 ringbiv 7.1578947 rodbiv 7.1578947
triv 2.6052632 ringquad 4.9473684 rodquad 11.263158
number 39 univ 2.7948718 ringbiv 7.0769231 rodbiv 7.1794872
triv 2.5384616 ringquad 5.1282051 rodquad 11.282051
number 40 univ 2.7500000 ringbiv 7.1000000 rodbiv 7.2000000
triv 2.5500000 ringquad 5.3000000 rodquad 11.100000
number 41 univ 2.7317073 ringbiv 7.2195122 rodbiv 7.1707317
triv 2.4878049 ringquad 5.3658536 rodquad 11.024390
number 42 univ 2.6666667 ringbiv 7.2857143 rodbiv 7.1428571
triv 2.4285714 ringquad 5.4285714 rodquad 11.047619
number 43 univ 2.6046512 ringbiv 7.3488372 rodbiv 7.1162791
triv 2.3720930 ringquad 5.4883721 rodquad 11.069767
number 44 univ 2.5681818 ringbiv 7.2727272 rodbiv 7.0454545
triv 2.3863636 ringquad 5.7272727 rodquad 11.000000
number 45 univ 2.5111111 ringbiv 7.2888889 rodbiv 6.9777778
triv 2.3333333 ringquad 5.8666666 rodquad 11.022222
number 46 univ 2.5000000 ringbiv 7.2173913 rodbiv 6.8260870
triv 2.4130435 ringquad 6.1739130 rodquad 10.869565
number 47 univ 2.4468085 ringbiv 7.4042553 rodbiv 6.7659575
triv 2.3617021 ringquad 6.2127659 rodquad 10.808511
number 48 univ 2.3958333 ringbiv 7.5000000 rodbiv 6.7916666
triv 2.3125000 ringquad 6.4166667 rodquad 10.583333
number 49 univ 2.3469388 ringbiv 7.6734694 rodbiv 6.7346938
triv 2.2653061 ringquad 6.4489796 rodquad 10.530612
number 50 univ 2.3000000 ringbiv 7.8000000 rodbiv 6.6400000
triv 2.2200000 ringquad 6.5600000 rodquad 10.480000

End of program

```
chiasma frequency in autotetraploids version 2b jan65;
begin integer array end[1:2,1:9,1:4];
          integer int1,int2,int3,n,y,z,x,w,q,one,two,posn,set,chrom,
                p,keyword,univ,rod,rod2,rod3,mult,mult2,rod4,
               mult3,mult4,rodbiv,triv,rodquad,ringquad,ringbiv,group,three,four;
          switch ss:=label1,label2,label3;
          procedure randomise(int1,int2);
integer int1,int2;
begin integer k,one,storac,storar,i1,i2;
switch sw:=iterate,exit,repeat;
k:=455470314;one:=1;
repeat:     elliott(3,0,keyword,0,5,2,k);
              elliott(2,0,storac,0,5,7,0);
            elliott(2,0,storar,0,0,0,0);
 iterate: elliott(3,0,storar,0,5,0,38);
            elliott(3,0,storac,0,0,0,0);
            elliott(5,4,7,0,1,6,keyword);
            elliott(5,0,7,0,5,7,0);
            elliott(1,0,keyword,0,2,0,storac);
           if storac=0 then goto exit;
          elliott(3,0,storac,0,0,0,0);
            elliott(0,4,keyword,0,5,2,one);
           elliott(2,0,storac,0,5,7,0);
           elliott(2,0,storar,0,0,0,0);
           goto iterate;
  exit: i1:=511; i2:=1536;
          elliott(3,0,keyword,0,2,3,i1);
          elliott(0,3,i2,0,5,1,9);
          elliott(2,0,i2,0,0,0,0);
            int1:=i1/7; int2:=i2+1;
            if int1=0 or int1=73 then goto repeat;
 end;
         univ:=rodbiv:=triv:=rodquad:=ringquad:=ringbiv:=0;
          keyword:=262143;
          elliott(0,6,0,0,7,0,0);
          elliott(2,3,keyword,0,0,0,0);

                    for n:=1 step 1 until 30 do

                 begin for w:=1 step 1 until 72 do

                    begin posn:=entier((w+35)/36);
                          if w>36 then set:=entier(((w-36)+3)/4)
                         else set:=entier((w+3)/4);
                         if w>36 then chrom:=((w-36)+4)-(set*4) else
                              chrom:=(w+4)-(set*4);
                     end[posn,set,chrom]:=0;   end;
          for z:=1 step 1 until 27 do
           begin label1: randomise(int1,int2);
                one:=two:=three:=four:=0;
                 if int1>36 then int1:=entier(((int1-36)+3)/4)
                else int1:=entier((int1+3)/4);
           for w:=1 step 1 until 4 do
                  begin if end[1,int1,w]=0 then one:=one+1 else two:=two+1;
            if end[2,int1,w]=1 then three:=three+1;
                        if end[2,int1,w]=2 then four:=four+1;
                   end;
                   if one=4 then end[1,int1,1]:=end[1,int1,2]:=1
               else     if two=2then end[1,int1,3]:=end[1,int1,4]:=2
               else   if two=4 then begin if end[2,int1,int2]>0 then goto label1;
                            if three=0 then
                                   begin chrom:=int2; group:=int1;
                     end[2,group,chrom]:=1;
                               label3:      randomise(int1,int2);
                                     if int2=chrom then goto label3 else
                                       end[2,group,int2]:=1; end
                               else if three=2 then begin
                group:=int1;   chrom:=int2;
                           end[2,group,chrom]:=2;
                                 for w:=1 step 1 until 4 do
                               begin if end[2,group,w]=0 then end[2,group,w]:=2;  end;
                                    end else if four=2 then goto label1; end; end;p:=1
                             for w:=1 step 1 until 72 do

                  begin posn:=entier((w+35)/36);
                         if w>36 then set:=entier(((w-36)+3)/4)
                        else set:=entier((w+3)/4);
                        if w>36 then chrom:=((w-36)+4)-(set*4) else
                             chrom:=(w+4)-(set*4);
              print end[posn,set,chrom];   end;
                   for q :=1 step 1 until 9 do
                    begin  rod:=rod2:=rod3:=mult:=mult2:=rod4:=
                        mult3:=mult4:=0; for w:=1 step 1 until 4 do
                  begin  if end[p,q,w]=0 and end[p*2,q,w]=0
                    then univ:=univ+1    else
                    if end[p,q,w]=0 and end[p*2,q,w]=1 then
                           rod:=rod+1    else
                  if end[p,q,w]=0 and end[p*2,q,w]=2 then
                  rod2:=rod2+1    else
                 if end[p,q,w]=1 and end[p*2,q,w]=0 then
                    rod3:=rod3+1   else
              if end[p,q,w]=1 and end[p*2,q,w]=1 then
                          mult:=mult+1  else
                 if end[p,q,w]=1 and end[p*2,q,w]=2 then
                   mult2:=mult2+1  else
                 if end[p,q,w]=2 and end[p*2,q,w]=0 then
                     rod4:=rod4+1  else
               if end[p,q,w]=2 and end[p*2,q,w]=1 then
                  mult3:=mult3+1  else
                 if end[p,q,w]=2 and end[p*2,q,w]=2 then
                   mult4:=mult4+1 ;
                   end;
                   print univ,sameline,rod,rod2,rod3,mult,mult2,rod4,mult3,mult4;
            if  rod=2 then rodbiv:=rodbiv+1;
                 if rod2=2 then rodbiv:=rodbiv+1;
                 if rod3=2 then rodbiv:=rodbiv+1;
                if rod4=2 then rodbiv:=rodbiv+1;
                 if rod=1 and mult=1 and rod3=1 then triv:=
                  triv+1;
                   if rod=1 and mult=1 and mult2=1 and rod2=1
                   then rodquad:=rodquad+1;
                  if rod3=1 and mult=1 and mult3=1 and rod4=1
                    then rodquad:=rodquad+1;
                 if mult=1 and mult3=1 and mult4=1 and  mult2=1
                  then ringquad:=ringquad+1;
                 if mult=2 then ringbiv:=ringbiv+1;
                 if mult2=2 then ringbiv:=ringbiv+1;
                 if mult3=2 then ringbiv:=ringbiv+1;
                 if mult4=2 then ringbiv:=ringbiv+1;  end;
     print £n?,sameline,n,£  ?;
     print £ringbiv?,sameline,(ringbiv*2)/(36*n),£  ?;
     print £rodbiv?,sameline,(rodbiv*2)/(36*n),£  ?;
     print £univ?,sameline,univ/(36*n),£
     ?;
     print £triv?,sameline,(triv*3)/(36*n),£  ?;
     print £ringquad?,sameline,(ringquad*4)/(36*n),£  ?;
     print £rodquad?,sameline,(rodquad*4)/(36*n),££L??;
     end; end; end;
```

chiasma frequency in autotetraploids version 2 jan65

Free Store = 20 to 5195

number 1 univ 2.0000000 ringbiv 4.0000000 rodbiv 22.000000
triv .00000000 ringquad .00000000 rodquad 8.0000000
number 2 univ 3.0000000 ringbiv 7.0000000 rodbiv 20.000000
triv .00000000 ringquad .00000000 rodquad 6.0000000
number 3 univ 3.3333333 ringbiv 6.6666667 rodbiv 18.000000
triv .00000000 ringquad .00000000 rodquad 8.0000000
number 4 univ 4.5000000 ringbiv 5.5000000 rodbiv 15.000000
triv .00000000 ringquad 3.0000000 rodquad 8.0000000
number 5 univ 4.8000000 ringbiv 5.2000000 rodbiv 14.000000
triv .00000000 ringquad 4.0000000 rodquad 8.0000000
number 6 univ 4.6666667 ringbiv 4.6666667 rodbiv 14.000000
triv .00000000 ringquad 4.6666667 rodquad 8.0000000
number 7 univ 4.8571428 ringbiv 4.5714286 rodbiv 13.428571
triv .00000000 ringquad 5.7142857 rodquad 7.4285714
number 8 univ 4.2500000 ringbiv 4.2500000 rodbiv 14.000000
triv .00000000 ringquad 5.5000000 rodquad 8.0000000
number 9 univ 4.2222222 ringbiv 4.4444444 rodbiv 14.000000
triv .00000000 ringquad 5.7777777 rodquad 7.5555556
number 10 univ 4.0000000 ringbiv 4.0000000 rodbiv 13.600000
triv .00000000 ringquad 6.0000000 rodquad 8.4000000
number 11 univ 4.0000000 ringbiv 4.3636364 rodbiv 13.090909
triv .00000000 ringquad 5.8181818 rodquad 8.7272727
number 12 univ 4.0000000 ringbiv 4.6666667 rodbiv 13.000000
triv .00000000 ringquad 6.0000000 rodquad 8.3333333
number 13 univ 4.0000000 ringbiv 4.7692308 rodbiv 12.461538
triv .00000000 ringquad 5.8461538 rodquad 8.9230769
number 14 univ 3.7142857 ringbiv 4.8571428 rodbiv 12.571428
triv .00000000 ringquad 5.4285714 rodquad 9.4285714
number 15 univ 3.4666667 ringbiv 4.5333334 rodbiv 12.800000
triv .00000000 ringquad 5.8666666 rodquad 9.3333333
number 16 univ 3.3750000 ringbiv 4.7500000 rodbiv 12.875000
triv .00000000 ringquad 6.0000000 rodquad 9.0000000
number 17 univ 3.2941177 ringbiv 4.7058824 rodbiv 12.470588
triv .00000000 ringquad 5.8823529 rodquad 9.6470588
number 18 univ 3.2222222 ringbiv 5.1111111 rodbiv 12.555555
triv .00000000 ringquad 5.7777777 rodquad 9.3333333

triv .00000000 ringquad 6.0000000 rodquad 9.0000000
number 17 univ 3.2941177 ringbiv 4.7058824 rodbiv 12.470588
triv .00000000 ringquad 5.8823529 rodquad 9.6470588
number 18 univ 3.2222222 ringbiv 5.1111111 rodbiv 12.555555
triv .00000000 ringquad 5.7777777 rodquad 9.3333333

number 19 univ 3.2631579 ringbiv 5.2631579 rodbiv 12.526316
triv .00000000 ringquad 6.1052631 rodquad 8.8421052
number 20 univ 3.2000000 ringbiv 5.3000000 rodbiv 12.700000
triv .00000000 ringquad 6.4000000 rodquad 8.4000000
number 21 univ 3.1428571 ringbiv 5.5238095 rodbiv 12.666667
triv .00000000 ringquad 6.4761904 rodquad 8.1904762
number 22 univ 3.2727273 ringbiv 5.8181818 rodbiv 12.363636
triv .00000000 ringquad 6.7272727 rodquad 7.8181818
number 23 univ 3.1304348 ringbiv 5.8260869 rodbiv 12.434783
triv .00000000 ringquad 6.7826087 rodquad 7.8260869
number 24 univ 3.0833333 ringbiv 5.7500000 rodbiv 12.000000
triv .00000000 ringquad 6.6666667 rodquad 8.5000000
number 25 univ 2.9600000 ringbiv 5.5200000 rodbiv 11.840000
triv .00000000 ringquad 6.7200000 rodquad 8.9600000
number 26 univ 2.8461538 ringbiv 5.6923077 rodbiv 11.923077
triv .00000000 ringquad 6.6153846 rodquad 8.9230769
number 27 univ 2.7407407 ringbiv 5.5555555 rodbiv 12.000000
triv .00000000 ringquad 6.8148148 rodquad 8.8888889
number 28 univ 2.7142857 ringbiv 5.4285714 rodbiv 11.714286
triv .00000000 ringquad 6.8571428 rodquad 9.2857143
number 29 univ 2.6206897 ringbiv 5.4482758 rodbiv 11.793103
triv .00000000 ringquad 6.8965517 rodquad 9.2413793
number 30 univ 2.5333333 ringbiv 5.7333333 rodbiv 11.866667
triv .00000000 ringquad 6.6666667 rodquad 9.2000000
number 31 univ 2.5806452 ringbiv 5.8064516 rodbiv 11.612903
triv .00000000 ringquad 6.8387097 rodquad 9.1612903
number 32 univ 2.5000000 ringbiv 5.8750000 rodbiv 11.625000
triv .00000000 ringquad 6.8750000 rodquad 9.1250000
number 33 univ 2.4242424 ringbiv 5.7575758 rodbiv 11.575758
triv .00000000 ringquad 7.0303030 rodquad 9.2121212
number 34 univ 2.4117647 ringbiv 5.8235294 rodbiv 11.529412
triv .00000000 ringquad 7.1764706 rodquad 9.0588235
number 35 univ 2.4000000 ringbiv 5.8285714 rodbiv 11.428571
triv .00000000 ringquad 7.3142857 rodquad 9.0285714
number 36 univ 2.3333333 ringbiv 5.7777777 rodbiv 11.444444
triv .00000000 ringquad 7.4444444 rodquad 9.0000000
number 37 univ 2.2702703 ringbiv 5.7297297 rodbiv 11.459459
triv .00000000 ringquad 7.6756757 rodquad 8.8648648
number 38 univ 2.2105263 ringbiv 5.7368421 rodbiv 11.421053
triv .00000000 ringquad 7.7894737 rodquad 8.8421052
number 39 univ 2.2051282 ringbiv 5.7948718 rodbiv 11.282051

number 36 univ 2.3333333 ringbiv 5.7777777 rodbiv 11.444444
triv .00000000 ringquad 7.4444444 rodquad 9.0000000
number 37 univ 2.2702703 ringbiv 5.7297297 rodbiv 11.459459
triv .00000000 ringquad 7.6756757 rodquad 8.8648648
number 38 univ 2.2105263 ringbiv 5.7368421 rodbiv 11.421053
triv .00000000 ringquad 7.7894737 rodquad 8.8421052
number 39 univ 2.2051282 ringbiv 5.7948718 rodbiv 11.282051

triv .00000000 ringquad 7.8974359 rodquad 8.8205128
number 40 univ 2.1500000 ringbiv 5.8000000 rodbiv 11.250000
triv .00000000 ringquad 8.0000000 rodquad 8.8000000
number 41 univ 2.1463415 ringbiv 5.7560976 rodbiv 11.121951
triv .00000000 ringquad 8.1951220 rodquad 8.7804878
number 42 univ 2.0952381 ringbiv 5.7142857 rodbiv 11.047619
triv .00000000 ringquad 8.3809524 rodquad 8.7619047
number 43 univ 2.0465116 ringbiv 5.8139534 rodbiv 11.023256
triv .00000000 ringquad 8.4651163 rodquad 8.6511628
number 44 univ 2.0000000 ringbiv 5.8181818 rodbiv 11.000000
triv .00000000 ringquad 8.6363636 rodquad 8.5454545
number 45 univ 2.0000000 ringbiv 5.9111111 rodbiv 10.844444
triv .00000000 ringquad 8.8000000 rodquad 8.4444444
number 46 univ 1.9565217 ringbiv 5.9565217 rodbiv 10.608696
triv .00000000 ringquad 8.7826087 rodquad 8.6956521
number 47 univ 1.9148936 ringbiv 6.0425532 rodbiv 10.510638
triv .00000000 ringquad 8.9361702 rodquad 8.5957447
number 48 univ 1.8750000 ringbiv 6.2083333 rodbiv 10.416667
triv .00000000 ringquad 9.0000000 rodquad 8.5000000
number 49 univ 1.8367347 ringbiv 6.1632653 rodbiv 10.285714
triv .00000000 ringquad 9.2244898 rodquad 8.4897959
number 50 univ 1.8000000 ringbiv 6.2800000 rodbiv 10.160000
triv .00000000 ringquad 9.3600000 rodquad 8.4000000

End of program

APPENDIX VI

The following programmes are written in Elliott 8-hole Algol and are suitable for running on an Elliott 803 computer.

Part of the programme is a random number generator and a large number to initiate the process has to be set up on the computer consol. This is fed in as for data.

Running time - approximately 50 minutes.

A P P E N D I X VII

SPECIES LISTS TAKEN AT LOCALITIES 1 AND 2 IN THE VALLÉE DE RIO ESERA, CENTRAL PYRENEES

APPENDIX VII

A species list taken at locality 2 in the Vallée de Rio Esera

Species	% Frequencies estimated by calculating the occurence in 100 randomly selected ½ sq. metre areas Estimated cover = 62%
Agrostis alba?	2%
Armeria plantaginea	7%
Arnica montana	1%
Carduus carlinifolius	5%
Carex verna	34%
Carlina acaulis	39%
Cotoneaster integerrimus	4%
Festuca ovina	37%
Galium verum	2%
Galium saxatile	4%
Genista purgans	11%
Geum pyrenaicum	3%
Lathyrus montanum	3%
Lotus corniculatus	4%
Pinus mugo	1%
Poa annua	40%
Polygonum alpinum	3%
Potentilla sterilis	18%
Rhododendron ferrugineum	1%
Rosa canina	25%
Sedum montanum	13%
Sempervivum tectorum	19%
Thymus serpyllum	49%
Veratrum album	22%
Verbascum spp.	1%
Veronica officinalis	47%
Viola riviniana	30%

APPENDIX VII

A species list taken at locality 1 in the Vallée de Rio Esera

Sesleria coerulea

Chrysanthemum leucanthemum

Sempervivens tectorum

Achillea millefolium

Lotus corniculatus

Thymus nervosum

Festuca ovina

Dianthus caryophyllus

Euphrasia sp.

Gentiana verna

Vicia pyrenaica

Anthyllis vulnerarioides

Sedum atratum

Alchemilla sp.

Helianthemum nummularium

Biscutella pyrenaica

Thymus serpyllum

Trifolium thallii

Polygala alpina

Poa alpina

Draba aizoides

Scabiosa velutina

APPENDIX VIII

A SPECIES LIST TAKEN AT THE LIMESTONE CLIFF LOCALITIES OF THE GREAT ORME AND HUMPHREY HEAD

APPENDIX VIII

A species list taken at the limestone cliff localities of the Great Orme and Humphrey Head

Achillea millefolium
Agrostis setacea
Allium shoenoprasum
Anthoxanthum odoratum
Anthyllis vulneraria
Armeria maritima
Bellis perennis
Briza media
Campanula rotundifolia
Carex flacca
Carex verna
Cerestium tetandrum
Cochlearia danica
Cynosorus cristatus
Deschampsia cespitosa
Euphrasia sp.
Restuca rubra
Festuca ovina
Galium saxatile
Galium verum
Geranium sanguineum
Helianthemum canum
Helianthemum nummularium
Hieracium pilosella
Holcus lanatus
Koelaria gracilis
Leontodon autumnalis
Linum catharticium
Lotus corniculatus
Plantago coronopus
Plantago lanceolata
Plantago maritima
Poa anna
Polygola vulgaris
Potentilla erecta
Poterium sanguisorba
Prunella vulgaris
Rumex acetosa
Scilla verna
Sedum anglicum
Sesleria caerulea
Sieglingia decumbens
Thymus drucei
Thymus serpyllum
Trifolium repens
Veronica champaedrys

APPENDIX IX

A SPECIES LIST TAKEN AT CWYM IDWAL

APPENDIX IX

A species list taken at Cwym Idwal

The locality in which Chrysanthemum leucanthemum L. was growing in Cwym Idwal contained a variety of ecological niches and this is reflected in the following species list.

Asplenium trichomanes
Agrostis canina
Anemone nemorosa
Anthoxanthum odoratum
Blech.num spicata
Deschampsia flexuosa
Deschampsia caespitosa
Drosera rotundifolia
Euphrasia sp.
Festuca rubra
Festuca ovina
Geum rivale
Hieracium sp.
Lycopodium selago
Lycopodium alpinum
Oxyria digyna
Pinguicula vulgaris
Potentilla erecta
Saxifraga aizoides
Saxifraga hynnoides
Saxifraga stellaris
Sedum rosea
Solidago virgaurea
Thalictrum alpinum
Thymus drucei
Vaccinium myrtillus
Viola riviniana

APPENDIX X

THE THIN LAYER CHROMATOGRAPHY METHOD USED

APPENDIX X

The Thin Layer Chromatographic Method used

Glass plates were spread with Silica Gel G using a Shandon plate spreader adjusted to 250 microns in depth. The Silica Gel G was prepared for spreading by rapidly mixing Silica Gel powder with distilled water in the proportion by weight of 1 : 2 respectively. Activation of the layer was obtained by heating the plates at 110°C for 25 minutes.

Extraction of leaf phenolic components was performed by drying basal leaf samples, grinding them up and then shaking the resulting powders with absolute Ethanol. Approximately 0.25 grms. of powdered leaves were mixed with 10 mls. of Ethanol. The mixture was permitted to stand overnight and then the solvent was 'spotted' onto the prepared plates. Normally, 10 drops of solvent were 'spotted' at each site.

Both one and two dimensional chromatograms were prepared. The solvents used were:-

(A) butanol : acetic acid : water
4 1 1

(B) ethyl acetate : methyl ethyl betone ; formic acid ; water
5 3 1 1

Solvent (B) was used for one dimensional chromatograms and the second phase of two dimensional chromatograms. Using this solvent, care had to be taken to ensure tank saturation by the solvent vapours before

the chromatogram could be started.

Solvent (A) was used for the first phase of the two dimensional chromatograms. It was noted that the solvents used were rather similar in their separating properties and it would have been preferable to have replaced solvent (A) by a more polar mixture such as

water	:	ethanol	:	methyl ethyl ketone	:	acetylacetone
13		3		3		1

(Randerath (1963)). However, time did not permit further experimentation.

On average, 45 minutes were required for the solvents to rise 100 cms.

Examination of the resulting spots was carried out in daylight and also under long wave-length ultra violet light. Records of the spot positions and colours were kept for later analysis.

A P P E N D I X XI

RANDOMISED GROWTH EXPERIMENT

APPENDIX XI

Randomised Growth Experiment

October 1964.

Mature plants, 300 in number, were removed from pots or garden beds, the soil carefully washed out of them and repotted into 6" pots containing John Innes No. 2 potting compost. The pots were then sunk up to their rims in a flower bed to prevent frosting and arranged in a random sequence. The pot centres were 18" apart on every side. The growth plot had a south-easterly aspect and sloped gently in that direction.

June - July 1965.

The plants were harvested by measuring immediately those characters which were changed by pressing such as leaf thickness and capitulum diameter. Examples of midstem, lower stem and basal leaves and stem with infloresence were then pressed separately for further analysis.

APPENDIX XII

METHODS AND ASSUMPTIONS USED IN THE MULTI-VARIATE ANALYSIS

APPENDIX XII

Methods and assumptions used in the multivariate analysis

1. A Q type of analysis, i.e. the examination of association between pairs of operational taxonomic units over all characters, was employed.

2. The index of association used was the Pearson Product Moment Correlation Coefficient.

3. Characters were given equal weight. Whilst it was intuitively felt desirable to weight characters indiscriminate weighting would probably have introduced more errors than giving equal weight. The only logical way to assess the weighting value of characters is to evaluate their relative contributions to within and between group variation by discriminant analysis. Unfortunately, this is a circular argument since the groups have to be established before weighting values can be attributed to characters.

4. Where a specimen was incomplete and had characters missing then such characters were disregarded in the computation of correlation coefficients between that specimen and any other. In practice this process resulted in a reduction of up to 7 variables.

5. Operational taxonomic units were clustered by a weighted pair group method. Sokal and Rohlf (1962) point out that this method of clustering produces least distortion of the multidimensional relationships between O.T.U's. Reduction of the matrix was carried out by calculating arithmetical means between pairs of correlation coefficients after transforming them to Fisher's Z.

The outline and rationale for this process is given in Sokal and Sneath (1963).

APPENDIX XIII

DETAILS ON THE USES AND SPECIFICATIONS OF THE NUMERICAL TAXONOMIC PROGRAMS

APPENDIX XIII

Use of the numerical taxonomic programs

The suite of programs was designed and written to provide a fully integrated system for multivariate analysis of data, whilst retaining an element of flexibility in the process.

The programs are written in K.D.F.9 Algol and are not suitable for running on any other machine, since the input and output routines used are particular to the English Electric-Leo-Marconi K.D.F.9.

The programs have been converted into efficient binary programs using the Kidsgrove compiler in its new segmented-algol form. The use of segmented algol means that the progams can be united into one long Algol program and all run in the same production run. Lack of time has prevented this latter development being carried out on the present programs.

Specifications

There are no unreasonable limitations on size using K.D.F.9 for a numerical taxonomic study since the core store will hold data sufficient for a 230 O.T.U. x 230 O.T.U. matrix analysis with up to a hundred characters per O.T.U. The flow diagram on page 104 shows how the programs are interrelated. Printouts of the programs are given on pages 206 to 214.

Program FAYCL01

5-hole to 8-hole Paper Tape Conversion

This program can be used for converting 5-hole data tapes to the

8-hole format for which there is an automatic parity check on reading the tapes into K.D.F.9.

Where information is unavailable a value of -1 is punched.

Input 1. Call tape

CASE NORMAL

CARRIAGE RETURN LINE FEED

K

FAYCL0101KPU;

PROGAREA 873

IN 5; 8.

OUT 8; L.

——

2. Data tape (5-hole)

t;n; (t is the number of O.T.U's and n is the number of characters)

$x_1;x_2;x_3;$ .... $x_n;$

$t_1;t_2;t_3;$ .... $t_n;$ —— ——

Output An 8-hole version of the data. A print out of the contents of the tape assuming that the values to be printed are within the range 999.000 to 0.001;

Should the program FAYCL02 be used subsequently and not FAYCL03, then the 5-hole data put into FAYCL01 has to be in a certain order and the value of n equal to the total number of unmodified characters required for FAYCL02. The character order and value of n are

apparent from an examination of the specifications of FAYCL02.

Time taken - approximately 1 minute for
a 150 x 60 matrix

Program FAYCL02 Raw to modified data conversion

This program is used for taking raw data and converting it to ratios where necessary.

Input (1) Call Tape

```
CASE NORMAL
CARRIAGE RETURN LINE FEED
K
FAYCL0200KPU;
PROGAREA 1412;
IN 8; L.
OUT L.
```

(2) Data tape

n2;	(number of modified characters)
n3;	(number of unmodified characters)
n;	(number of unmodified characters)
t;	(number of O.T.U.'s)
w1;	(number of modified characters)
z;	(number of characters which do not require modifying)

q; (number of characters which are to be reduced to ratios)

t; (number of characters which are to be reduced by summation)

qr; (number of characters which are to be reduced by summation and ratios)

Data tape produced as output from FAYCL01;

— —

Comment A typical data tape might be of the following form:=

$9;16;16;20;9;3;8;2;3;A_1;B_1;C_1;D_1;E_1;F_1;G_1;H_1;J_1;K_1;L_1;M_1;N_1;O_1;P_1;$

$A_2;B_2;C_2;D_2;E_2;\ \ldots\ O_2;P_2;$

$A_3;B_3;C_3;D_3;E_3;\ \ldots\ O_3;P_3;$

$A_{20};B_{20};C_{20};D_{20};E_{20};\ \ldots\ O_{20};P_{20};$

The program would modify the data as follows:=

$A_x;\ B_x;\ C_x;\ D_x;\ E_x;\ F_x;\ G_x;\ H_x;\ I_x;\ J_x;\ K_x;\ L_x;\ M_x;\ N_x;\ O_x;\ P_x;$

$\downarrow$

$A_x;\ B_x;\ C_x;\ Dx/Ex;\ Fx/Gx;\ Hx/Ix;\ Jx/Kx;\ Lx + Mx;\ Px/Nx + Ox;$

Output (1) The modified data is transferred in binary form to a magnetic tape ready for input to FAYCL04

(2) A print out of the modified character values.

Time taken - approximately 50 seconds for a 150 x 60 matrix.

Program FAYCL0300APU

Direct Input

This program has been placed in the suite for use on small quantities of data where the tedium of converting raw data into ratios manually is negligible.

The relationship of this program to the rest of the suite can be seen in the flow diagram on page 186 . The program is used to put the data onto magnetic tape suitable for input to FAYCL04. Where information is not available a value of -1 is punched. A magnetic tape labelled PEARSON1 is required.

Input 1. Program tape

No call tape required for this program. The length of the program does not warrant translating into an efficient binary program. The program can be translated on the Walgol compiler KMW02.

2. Data tape

t; (number of O.T.U's)

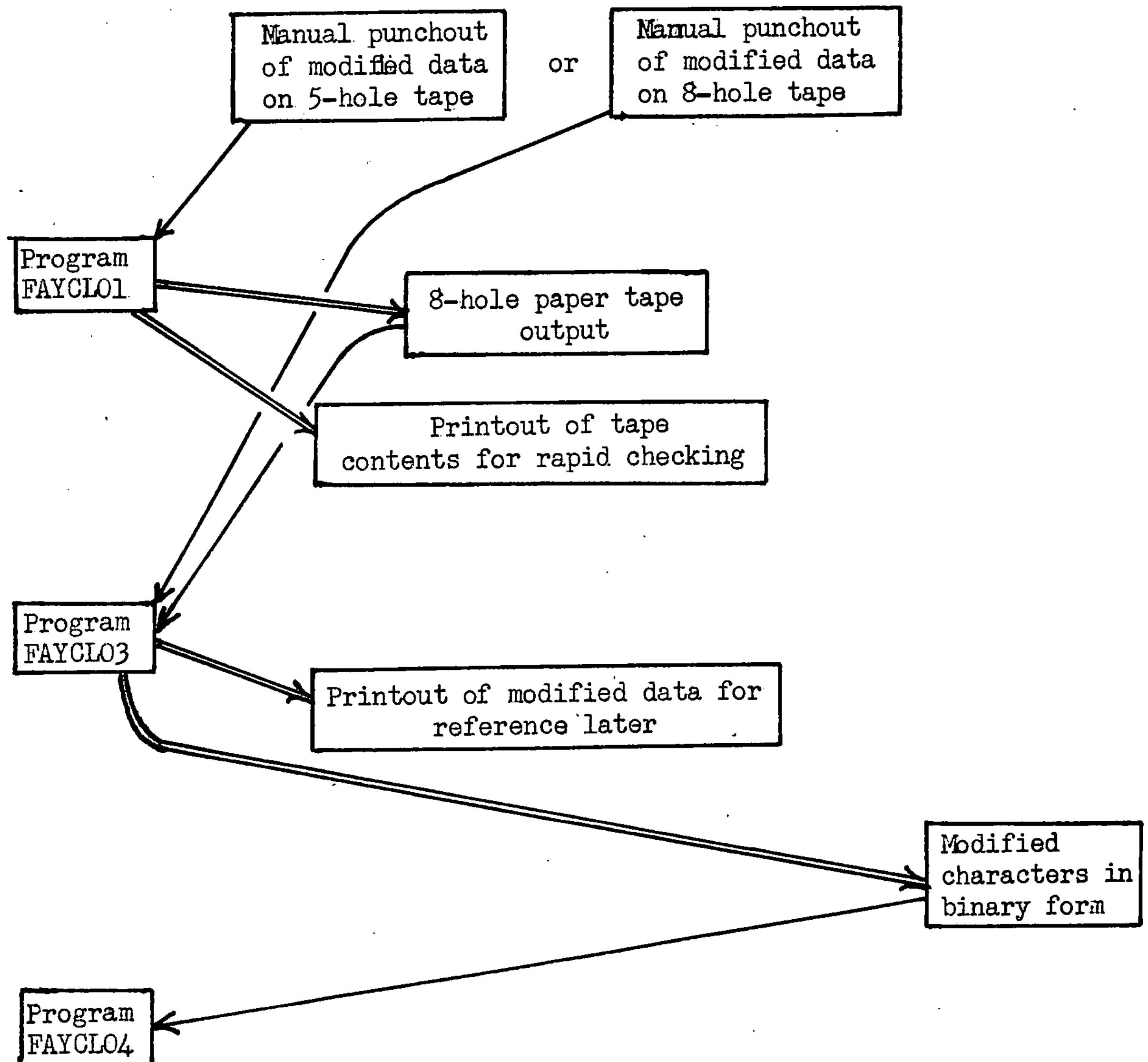
n; (number of characters)

$A_1;B_1;C_1;D_1;E_1;F_1;G_1;$ ....$n_1;$

$A_2;B_2;C_2;D_2;E_2;F_2;G_2;$ ....$n_2;$

$A_t;B_t;C_t;D_t;E_t;F_t;G_t;$ ....$n_t;$

Time taken := negligible. Normally 45 seconds


K.D.F9
computer
Manhandling routines involving
input and output
Magnetic tape
unit
Manual punchout
of modified data
on 5-hole tape
or
Manual punchout
of modified data
on 8-hole tape
Program
FAYCL01
8-hole paper tape
output
Printout of tape
contents for rapid checking
Program
FAYCL03
Printout of modified data for
reference later
Modified
characters in
binary form
Program
FAYCL04

Program FAYCL0404KPU

Product moment correlation coefficients, and taxonomic distance

This program is really the heart of the matter and computes product moment correlation coefficients and taxonomic distances between all O.T.U.'s. Comparisons are only made using characters for which there is information. The data is standardised by the program. A magnetic tape labelled PEARSON1 is required.

Input 1. Call tape

```
CASE NORMAL
CARRIAGE RETURN LINE FEED
K
FAYCL0404KPU;
PROGAREA 1799;
IN 8; L.
OUT L.
```

2. Data tape

t; (number of O.T.U.'s)

n; (number of characters)

Output 1. A print out of correlation coefficients, taxonomic distances and the identifiers of O.T.U.'s.

2. A binary form of the coefficients and identifiers of the

O.T.U's involved is transferred to a magnetic tape ready for input to FAYCLO504KPU;

Time taken := approximately 4 minutes for a 60 O.T.U. x 60 O.T.U. matrix.

Program FAYCLO504KPU;

Weighted pair cluster analysis

This program groups O.T.U's into clusters by joining the two most highly correlated O.T.U's or clusters at each cycle either into an existing cluster or into a new cluster. Clusters are numbered from 300 upwards so that on the first cycle the two most highly correlated O.T.U's will initiate cluster 300. Magnetic tapes labelled PEARSON1 and * * * * * * * * are required.

Input 1. Call tape

CASE NORMAL

CARRIAGE RETURN LINE FEED

K

FAYCLO504KPU;

PROGAREA ;

IN 8; L.

OUT L.

2. Data tape

a; (number of coefficients. This can be estimated according to the formula $a = \frac{t^2}{2} - \frac{1}{2}t$ where t is the number of O.T.U's.

n; number arrays. This is equal to $\frac{a}{528}$ rounded off to the next whole number.

Output A print out of the value of coefficient at which O.T.U's join, the identifiers of the O.T.U's concerned, and the cluster which they join or initiate, is obtained.

Program FAYCLO602KPU;

8-hole data output for input to discriminant function analysis KQX715013UPU

A visual examination of the results from FAYCLO504KPU should reveal what clusters the O.T.U's are grouped into. Should it be necessary to recognise those particular characters and character values which discriminate between any two clusters then FAYCLO6 can be used to extract the data of the two clusters in a form suitable for input to discriminant function program KQX715013UPU. A magnetic tape labelled PEARSON1 is required.

Input 1. Call tape

CASE NORMAL

CARRIAGE RETURN LINE FEED

K

FAYCLO602KPU;

PROGAREA 1324;

IN 8; L.

OUT L.

2. Data tape

n; (number of characters)

t; (total number of O.T.U's in two clusters to be analysed)

q; (number of O.T.U's in group 1)

qr; (number of O.T.U's in group 2)

Output an 8-hole paper tape punchout of the data in a form suitable for input to KQX715013UPU.

Comment A development of FAYCL0602APU, namely FAYCL0800APU is available. This program incorporates a facility for missing out characters during the data extraction so that the discriminant analysis is carried out on a selection of the characters taken from the characters available. For example, chromosome count and pollen grain diameter can be missed out. Options for leaving out up to ten characters are included.

Input 1. Call tape

CASE NORMAL

CARRIAGE RETURN LINE FEED

K.

FAYCL0800KPU;

PROGAREA ;

IN L; 8.

OUT L.

2. Data tape

n;	(number of characters)
t;	(number of O.T.U's)
q;	(number of O.T.U's in group 1)
qr;	(number of O.T.U's in group 2)
x1;	
x2;	
x3;	numbers of characters to be missed out in numerical order. If less than ten characters are to be missed out then a value of 200 is inserted instead e.g. if characters 7 and 13 are to be missed out, then x1 := 7, x2 := 13 and x3 to x 10 := 200
x4;	
x5;	
x6;	
x7;	
x8;	
x9;	
x10;	

Z_1; Z_2; ...... Zq; (identifiers of O.T.U's in group 1 in numerical order)

Y_1; Y_2; ...... Yqr; (identifiers of O.T.U's in group 2 in numerical order)

Output An 8-hole paper tape punch out of the data in a form suitable for input to KQX715013UPU. The data is produced on two separate tapes corresponding to group 1 and group 2 characters respectively.

Program KQX715013UPU

Discriminant function analysis

This program has been developed by M. Ellson of English Electric.

Subsequent corrections have been made. The program produces a pair of linear equations to discriminate between two groups or clusters of O.T.U's. Individual observations are then substituted into these equations to enable the user to decide to which population each individual belongs. The program provides a choice of doing a complete analysis at one time or of stopping before individual observations are read in and completing the analysis at a later date. A numerical breakpoint between the two groups is provided.

Input Input data is punched on 8-hole paper tape, i.e. as produced by either FAYCL06 or FAYCL08.

The number of variables can be up to 99 and the number of observations is limited only by the size of the store.

Observations are read in as matrices, one matrix for each group.

Output Results are output to the line printer. If section 1 only is required initially information is punched and on paper tape, to be read in again by section 2 at a later date.

The program checks each group matrix to ensure it has the required number of variables.

Input 1. Call tape.

CASE NORMAL

CARRIAGE RETURN LINE FEED

M

KQX715013UPU

2. Data tape

S; (matrix store required)

Customer name (up to 64 chars)

Job title (up to 64 chars)

2; (indicating that both sections are required)

2; (number of groups)

V; (number of characters)

Matrices of O.T.U's

Each matrix is one group and is punched as follows:

A; 1; 2; P; V; (P is the number of O.T.U's inthe first group)
(V is the number of characters in the first group)

Data tape for group 1 as produced by either FAYCL06 or FAYCL08

B; 1; 2; q; V; (q is the number of O.T.U's in the second group)
(V is the number of characters in the second group)

Data tape for group 1 as produced by either FAYCL06 or FAYCL08.

Matrix of individual O.T.U's

Data tape for individual observations produced by either FAYCL06 or FAYCL08 or punched by hand. See comments below

---

2. If section 1 only is required, initially data will be as follows:

S; (matrix area required)

Customer name

Job title

1;

ø;

2;

v;

as above

3. If section 2 only is required data will be as follows:

S;

Customer name

Job title

ø;

2;

Paper tape obtained by having previously used section 1.

Matrix of individual O.T.U's as specified in 1.

<u>Output</u>

1. If both sections are run the output will be:

Means of both groups

Variance-covariance matrix

Inverse variance-covariance matrix

Matrix of discriminant equations

Matrix of results of substituting individual values

2. If section 1 only is run output will be:

as above

as above

as above

as above

paper tape output of discriminant equations.

3. If section 2 only is run output will be:

Matrix of results of substituting individual values.

Comments If individual O.T.U's already used in the analysis are to be examined for their entry to either group then the data can be extracted by either FAYCL06 or FAYCL08. In any other case the data would have to be punched by hand.

APPENDIX XIV

CHARACTERS USED IN THE MULTIVARIATE ANALYSIS

APPENDIX 14

Characters used in the multivariate analysis, and the scoring adopted for each character

	Character	Score
1.	Time of flowering	Starts flowering before June 8th := 1 Starts flowering between June 8th and June 17 := 2 Starts flowering after June 17th := 3
2.	Mean number of branches per stem	As calculated
3.	Position of branching	Top of stem := 1 Midstem := 2 Base of stem := 3
4.	Height of plant	As measured
5.	Diameter of capitulum (includes rays on outer florets)	As measured
6.	Length of rays on outer florets	As measured
7.	Mean diameter of pollen	As measured
8.	Colour of margin of involucral bracts	Black := 1 Dark brown := 2 Mid brown := 3 Light brown or fawn := 4 Colourless := 5
9.	Chromosome number	As counted
10.	Length of hairs on mid stem	As measured
11.	'Naturality' of habitat	Habitat natural := 1 Habitat disturbed := 2
12.	Shape of ends of rays on outer florets. See Fig. XIV A Page 201.	As Calculated

	Characters	Score	
13.	Density of stem hairs as base of stem	Glabrous	:= 1
		Sparsely haired	:= 2
		Densely haired	:= 3
		Very densely haired	:= 4
14.	Colour of leaves N.B. colour checking was carried out using R.H.S. colour charts and freshly picked stem leaves	Very light green	:= 4
		Light green	:= 5
		Mid green	:= 6
		Dark green	:= 7
		Very dark green	:= 8
15.	Type of dentition of leaf margin of basal leaves. See Fig. XIV B	As calculated	
16.	Number of incisions in basal leaves $\frac{1}{4}$ width of leaf	As counted	
17.	Thickness of leaf lamina of basal leaf, measured midway between the main vein and edge of lamina. Measured using a micrometer screw gauge.	As measured	
18.	Regularity in depth of basal leaf incisions	Regular	:= 1
		Intermediate	:= 2
		Irregular	:= 3
19.	Dentition of basal leaves opposite or not opposite	Opposite	:= 1
		Intermediate	:= 2
		Not opposite	:= 3
20.	Length of midstem leaf	As measured	
21.	Number of midstem leaf incisions $\frac{1}{4}$ width	As counted	
22.	Type of dentition on leaf margin of midstem leaves. See Fig. XIV B	As calculated	
23.	Regularity in depth of midstem leaf incisions	Regular	:= 1
		Intermediate	:= 2
		Irregular	:= 3
24.	Dentition of midstem leaves opposite or not opposite	Opposite	:= 1
		Intermediate	:= 2
		Not opposite	:= 3

	Characters	Score
25.	Number of auricles at the base of midstem leaves	As counted
26.	Condition of pappus on outer achenes See Fig. XIV C	As calculated
27.	Thickness of stem	As measured
28.	The following characters are primarily concerned with shape rather than size and mainly consist of ratios.	
28.	Shape of a lower stem leaf lamina $Al/_{Bl}$. See Fig. XIV E	As calculated
29.	Ratio of deepest incision to maximum width in a lower stem leaf lamina. $Bl/_{Dl}$ See Fig. XIV E	As calculated
30.	Position of widest point in a lower stem leaf lamina. $Al/_{Cl}$ See Fig. XIV E	As calculated
31.	Proportion of the total number of incisions which are $\frac{1}{4}$ width of stem leaf. $Is/_{Hs}$. See Fig. XIV F	As calculated
32.	Number of incisions per unit length of midstem leaves. $Is/_{As}$. See Fig. XIV F	As calculated
33.	Position of narrowest point of mid stem leaf. $As/_{Gs}$. See Fig. XIV F	As calculated
34.	Ratio of maximum distance between incisions and midstem leaf length $As/_{Ns}$ See Fig. XIV F	As calculated
35.	Shape of midstem leaf (length/width) $As/_{Bs}$ See Fig. XIV F	As calculated
36.	Position of deepest incision in basal leaves. $Bb/_{Db}$. See Fig. XIV D	As calculated

	Characters	Score
37.	Shape of largest auricle on midstem leaf $Ps/_{Rs}$ See Fig. XIV F	As calculated
38.	Ratio of auricle length to width of leaf base. $Ps/_{Os}$ See Fig. XIV F	As calculated
39.	Ratio of width of midstem leaf base to maximum width of midstem leaf. $Os/_{Bs}$ See Fig. XIV F	As calculated
40.	Ratio of maximum width to narrowest width of midstem leaf. $Bs/_{Fs}$ See Fig. XIV F	As calculated
41.	Ratio of maximum width to deepest incision of midstem leaf. $Bs/_{Es}$ See Fig. XIV F	As calculated
42.	Position of widest point of midstem leaf. $Cs/_{Ds}$ See Fig. XIV F	As calculated
43.	Ratio of width to length of stem leaf teeth $Vs/_{Ts}$ See Fig. XIV F	As calculated
44.	Ratio of distance between midstem nodes and the height of the plant	As calculated
45.	Ratio of distance from capitulum to 6th node and height of the plant N.B. This ratio is used to differentiate those plants with short lengths between lower stem nodes from those with normal lengths between lower stem nodes. ~~See Fig. XIV H~~	As calculated
46.	Length of basal leaf lamina Bb + Cb See Fig. XIV D	As calculated
47.	Shape of basal leaf lamina Ab/Bb + Cb See Fig. XIV D	As calculated
48.	Ratio of length of petiole to length of lamina on basal leaves Eb/Bb + Cb See Fig. XIV D	As calculated

	Characters	Score
49.	Ratio of maximum distance between incisions and length of lamina in basal leaves. Nb/Bb + Cb See Fig. XIV D.	As calculated
50.	Shape of rays on outer florets Length ray/width ray	As calculated
51.	Number of rays on outer florets	As counted

N.B. Lack of material prevented achene size and certain of the phenolic pigments to be included in the analysis.

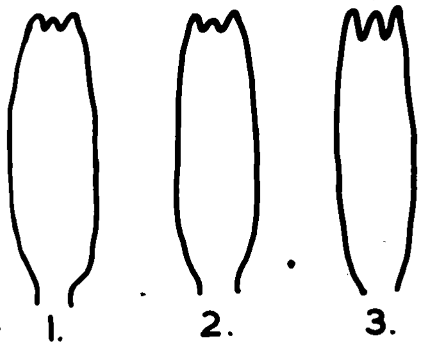


fig. XIVA

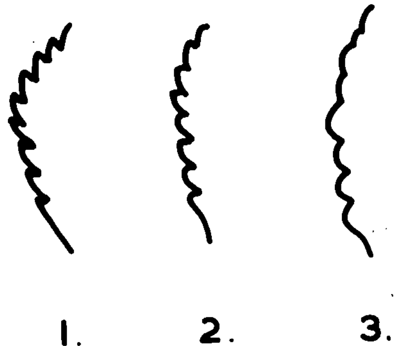


fig. XIVB

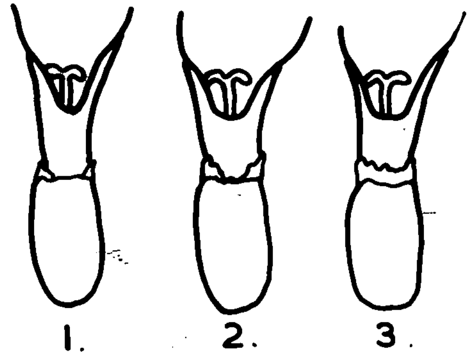
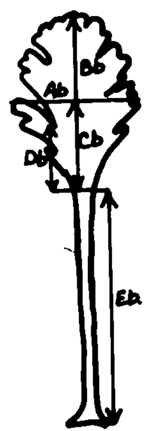
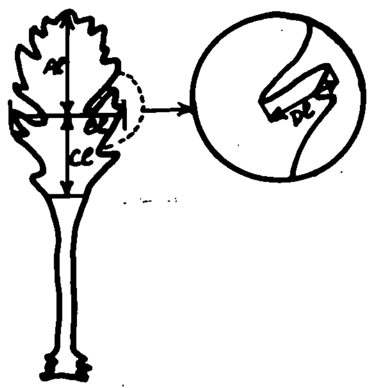


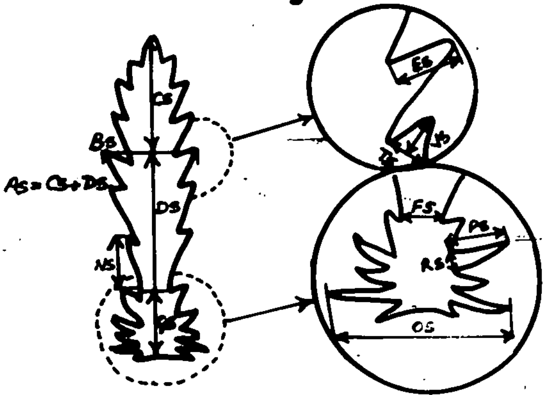
fig. XIVC



basal leaf
fig. XIVD



lower-stem leaf
fig. XIVE



mid-stem leaf
fig. XIVF

APPENDIX XV

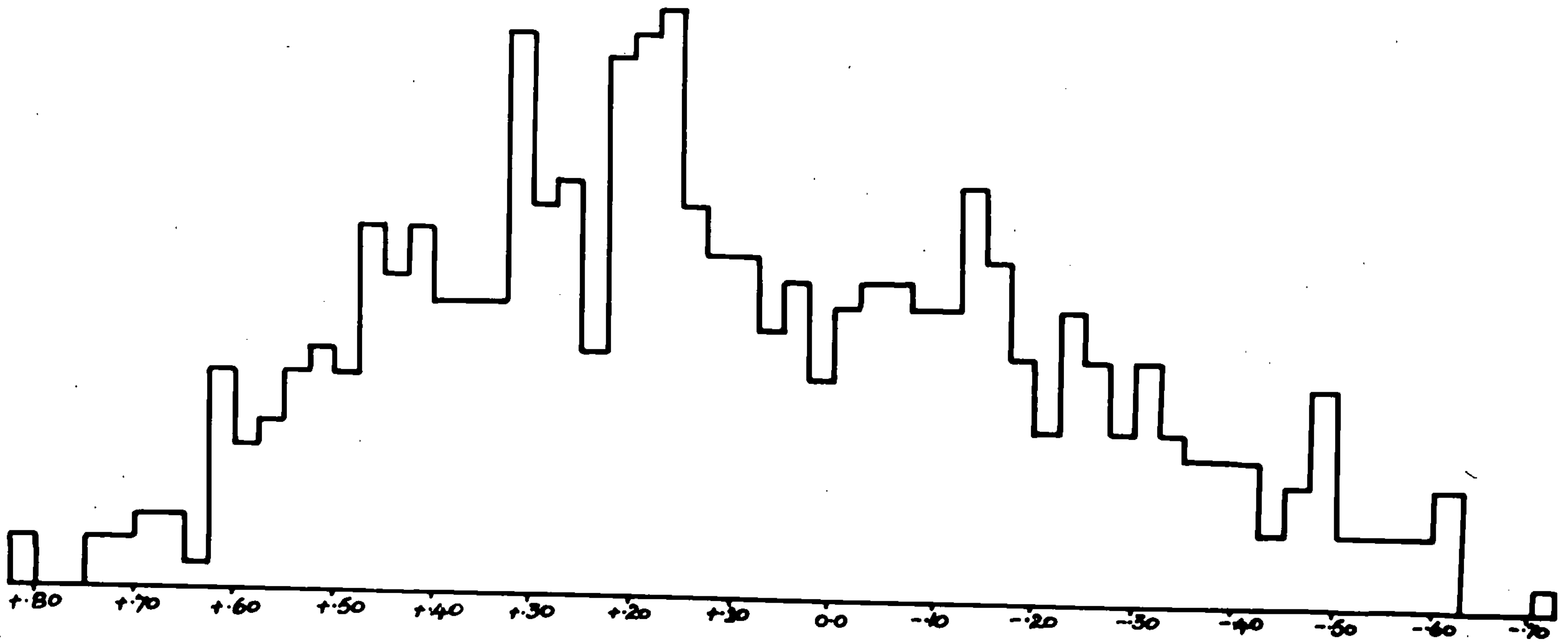
STATISTICAL SIGNIFICANCE AND DISTRIBUTION PROPERTIES OF THE CORRELATION COEFFICIENT WHEN USED IN NUMERICAL TAXONOMY

APPENDIX XV

The statistical significance of the product moment correlation coefficient when used in numerical taxonomy

Sokal and Sneath (1963) have pointed out that normal tests of significance cannot be applied to correlation coefficients when used for numerical taxonomy, because of the heterogeneity of the data. Normally, two variables can be regarded as being significancly correlated if their correlation is larger than + or - 0.25 when based upon 50 or more observations. When applied to numerical taxonomy, the level of significance at comparable correlation values would probably have to be lowered since all the observation values are not independent of each other. Unfortunately, there are no methods, other than empirical, of assessing by how much the confidence limits would have to be lowered.

A curious feature of the frequency distributions of correlation coefficients derived from the present study and that of Sokal and Rohlf (1962) are that they are positively skewed. See Fig. on page 203. Transformation to Fisher's Z does not compensate for the skewness of the distribution. Sokal and Sneath (1963) have attempted to explain such a positive skewness by invoking a concept of the impossibility of having an antiorganism, i.e. of having a high negative correlation between organisms. In situations employing standardised characters there seems to me no prima facie reason for suggesting that skewness of


frequency distribution of
correlation coefficients
+·80
+·70
+·60
+·50
+·40
+·30
+·20
+·10
0·0
-·10
-·20
-·30
-·40
-·50
-·60
-·70

distribution is due to an antiorganism concept. A more probable explanation is that the expected frequencies for positive and negative correlation coefficients are rarely equal when based upon samples of unequal sizes e.g. consider two samples of sizes A and B taken from two morphologically different populations such that the *between*-sample correlation coefficients are negative and the *within*-sample correlation coefficients are positive. The number of positive and negative correlations produced can be represented by:-

$$0.5\,(A^2 - A + B^2 - B) = \text{number of positive correlations}$$

$$AB = \text{number of negative correlations}$$

For a normal distribution of correlation coefficients, the ratio of

$$\frac{0.5(A^2 - A + B^2 - B)}{AB} = 1.$$

In a situation where A = B, then substituting for B produces a ratio of $\frac{A-1}{A}$. This ratio is less than one and the distribution is negatively skewed.

Similarly, where A = 2B, i.e. the size of one sample is twice that of the other, substitution for B produces a ratio of $\frac{1.5A - 1.5}{A}$. This ratio, employing usual values of A, would be much greater than one, and the distribution positively skewed.

APPENDIX XVI

SOME MORPHOLOGICAL COMPARISONS MADE BETWEEN 4 POPULATIONS

APPENDIX XVI

Certain characters have been compared between two 'diploid' and two 'tetraploid' populations. Parameters were measured on 25 plants from each population. Only some of the plants from each population had been cytologically identified.

	X1 Malham Moor (4n)	X2 Fen End Warwickshire (4n)	X3 Bearpark Durham (2n)	X4 Loggerheads Flintshire (2n)
Mean pollen grain diameter. Std. deviation	34.2 μ ± 2.8	33.8 μ ± 3.1	30.3 μ ± 4.7	29.8 μ ± 3.7
Mean number of branches. Std. deviation	1.60 ± 0.39	1.43 ± 0.41	0.95 ± 0.35	0.46 ± 0.33
Mean number of auricles on mid stem leaves. Std. deviation.	3.89 ±1.89	5.13 ±0.78	6.61 ±0.51	7.43 ±0.83
Mean width of mid stem leaves. Std. deviation.	1.43 cms. ±0.16	1.01 cms. ±0.09	0.82cms. ±0.06	0.85cms. ±0.04
Ratio of depth of deepest incision to width of basal leaves. Std. deviation.	4.67 ±0.63	4.47 ±0.54	4.32 ±0.62	2.54 ±0.46

T - TESTS & SIGNIFICANCE

POLLEN GRAIN DIAMETER

	X1	X2	X3	X4
X1		0·49 n.s.	3·56 0·01	4·74 0·001
X2			3·10 0·01	4·14 0·001
X3				0·41 n.s.
X4				

NUMBER OF BRANCHES

	X1	X2	X3	X4
X1		1·50 n.s.	6·19 0·001	11·15 0·001
X2			4·45 0·001	9·21 0·001
X3				5·09 0·001
X4				

NUMBER OF AURICLES

	X1	X2	X3	X4
X1		4·62 0·001	11·30 0·001	12·90 0·001
X2			7·94 0·001	10·09 0·001
X3				4·20 0·001
X4				

WIDTH OF MIDSTEM LEAVES

	X1	X2	X3	X4
X1		11·43 0·001	17·84 0·001	17·58 0·001
X2			8·78 0·001	8·12 0·001
X3				2·08 n.s.
X4				

DEEPEST INCISION / WIDTH OF LEAF

	X1	X2	X3	X4
X1		1·20 n.s.	1·98 n.s.	13·65 0·001
X2			0·91 n.s.	13·60 0·001
X3				11·52 0·001
X4				

N.S. = NOT SIGNIFICANT.

APPENDIX XVII

PROGRAM AND DATA PRINTOUTS OF THE PROGRAMS USED IN THE MULTIVARIATE ANALYSIS

```
→ESTABLISH FAYCLO100APU;FIVE TO EIGHT HOLE CONVERSION PEAR1;O/PL;→
begin library A1,A5,A4,A12;   integer x,y,n,t,z,F1,F2;
       real w;
       real array cu[1:100];
       open(20);open(30);open(10);
       F1:=format([ss-ndd.dd;]);
       F2:=format([ss-ndd.dd;c]);
       t:=read(20); n:=read(20);
comment t=number of otus. n=number of characters;
       for x:=1 step 1 until t do
begin for z:=1 step 1 until n do
     begin cu[z]:=read(20);
     end;
      y:=0;
       for z:= 1 step 1 until n do
     begin if y>8 then begin
           w:=cu[z]; y:=0; write(30,F2,w); write(10,F1,w);
                        end
          else begin w:=cu[z]; y:=y+1; write(30,F1,w); write(10,F1,w);
               end;
     end; write text(30,[[4c]]); gap(10,30);
 end;
 close(20); close(30); close(10);
end→
```

FAYCL0[illegible] [illegible]2/1[illegible]/66
STR3[illegible]

1.00	[illegible]	[illegible]	38.00	2.45	0.85	9.00	20.00	36.00	[illegible].00
[illegible]	-1.[illegible]	1.00	3.00	3.00	142.00	2.00	0.00	-1.00	1.00
[illegible]	[illegible]	3.75	0.00	1.00	50.00	1.00	1.00	3.50	0.50
0.42	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	10.00	0.00	10.00
3.70	3.70	[illegible].50	3.70	1.90	3.70	1.00	1.25	0.18	0.25
[illegible].10	[illegible].25	[illegible].77	0.77	1.00	1.00	0.35	1.00	0.12	1.35
2.40	10.20	1.60	1.90	-1.00	2.30	38.00	-1.00	38.00	38.00
0.48	1.25	1.00	1.60	2.00	0.32	0.85	-1.00	32.00	

[illegible]	[illegible]	[illegible]	61.00	3.30	1.00	9.70	3.00	36.00	9.00
[illegible]	-1.[illegible]	1.00	3.00	3.00	82.00	4.00	0.00	-1.00	2.00
[illegible]	[illegible]	[illegible].20	0.00	3.00	49.00	1.00	2.00	6.00	2.00
[illegible]	[illegible]	-1.00	-1.00	-1.00	-1.00	-1.00	10.00	0.00	10.00
4.20	[illegible]	1.20	4.20	1.15	3.70	1.72	0.72	0.15	0.35
[illegible]	[illegible].35	1.32	1.32	1.72	1.72	0.67	1.72	0.27	1.75
[illegible]	7.75	[illegible]	1.15	-1.00	2.80	61.00	-1.00	61.00	61.00
[illegible]	[illegible].72	[illegible].60	1.05	0.70	0.25	1.00	-1.00	32.00	

```
→ESTABLISH FAYCL0200APU;RAW TO MODIFIED DATA CONVERSION PEAR2;O/PL;→

begin library A1,A5,A4,A15,A7,A9;

 integer n2, n3; open(20); n2:=read(20); n3:=read(20);
comment n3 is upper limit of array for holding unmodified characters.
        n2 is upper limit of array for holding modified characters;
begin integer n,t,w1,z,q,r,x,d,y,s,F1,F2;
        real a,w,b;
       real array cu[1:n3], dv[1:n2];
    open(30); find(100,[PEARSON1]);
        n:=read(20); t:=read(20); w1:=read(20); z:=read(20); q:=read(20);
        r:=read(20); F1:=format([ss-ndd.dd;]); F2:=format([ss-ndd.dd;c]);
       interchange(100);

comment t=number of otus. n=total  number of unmodified characters
which have not been converted to ratios. q=number of characters
which have been converted to ratios. r=number of unmodified
characters which are summed. w1=total number of modified characters;

       for y := 1 step 1 until t do
       begin s:=0;
           for x:= 1 step 1 until n do
           begin cu[x]:=read(20); end;
          for x:= 1 step 1 until z do
          begin dv[x]:=cu[x]; end; d:=z;
         for x:= z+1 step 2 until z+q -1 do
         begin d:=d+1; w:=cu[x]; b:=cu[x+1];
         if w<0 then dv[d]:=w else begin if b<0 then
         dv[d]:=b else begin if b =0 then b:=0.01;  dv[d]:=w/b; end; end;
         end;
         x:= z+q+1; d:=d+1; w:=cu[x]; b:=cu[x+1];
               if w<0 then dv[d]:=w else begin if b<0 then dv[d]:=b else
               dv[d]:=a:=w+b; end;
         for x:= z+q+r+1 step 1 until z+q+r+3 do
         begin d:=d+1; w:=cu[x]; if w<0 then dv[d]:=w else begin if a=0
         then a:=0.01;
            dv[d]:=w/a;end; end;
            x:=z+q +r+3;
           w:=cu[x+1]; b:=cu[x+2];  if w<0 then dv[d+1]:=w else begin if
         b<0 then dv[d+1]:=b else begin if b=0 then b:= 0.01; dv[d+1]:=
         w/b;
            end; end;    dv[d+2]:=cu[x+3];
          write binary(100,dv,[modify]);
          for x:= 1 step 1 until w1 do
           begin if s>8 then begin w:=dv[x]; s:=0; write(30,F2,w); end
                 else begin w:=dv[x]; s:=s+1; write(30,F1,w); end;
            end;        write text(30,[[4c]]);
         end;    close(20); close(30); close(100);
end;
end→
```

```
FAYCLO200KPU   16/12/66
 STR30

   1.00;    4.00;    2.00;   38.00;    2.45;    0.85;    9.00;   20.00;   36.00;    8.00;
   2.00;   -1.00;    1.00;    3.00;    3.00;  142.00;    2.00;    0.00;   -1.00;    1.00;
   1.00;   90.00;    3.75;    0.00;    1.00;   50.00;    1.00;    1.00;    3.50;    0.50;
   0.42;   -1.00;   -1.00;   -1.00;
£+1.0000 0000 000 .+ 3;
                                                2.70;    7.40;    1.95;    3.70;    6.94;
   2.50;    0.32;    0.77;    2.86;    8.33;    6.56;    6.38;   -1.00;    0.06;   -1.00;
  79.17;    2.25;    0.71;    0.89;    0.14;   -1.00;   32.00;


   1.00;    3.00;    2.00;   61.00;    3.30;    1.00;    9.70;    3.00;   36.00;    9.00;
   2.00;   -1.00;    1.00;    3.00;    3.00;   82.00;    4.00;    0.00;   -1.00;    2.00;
   2.00;  118.00;    4.20;    0.00;    3.00;   49.00;    1.00;    2.00;    6.00;    2.00;
   0.39;   -1.00;   -1.00;   -1.00;
£+1.0000 0000 000 .+ 3;
                                                2.38;    3.50;    3.65;    2.15;    4.80;
   2.06;    0.27;    0.77;    2.57;    6.37;    0.73;    7.38;   -1.00;    0.05;   -1.00;
 160.53;    1.32;    0.80;    0.53;    0.19;   -1.00;   32.00;


   1.00;    2.00;    2.00;   31.00;    4.40;    1.45;    8.00;    1.00;   18.00;    0.70;
   1.00;   -1.00;    3.00;    1.00;    1.00;  125.00;    4.00;    0.00;   -1.00;    3.00;
   3.00;  130.00;    4.40;    1.70;    3.70;   49.00;    1.25;    3.00;    7.50;    0.50;
   0.43;   -1.00;   -1.00;   -1.00;    5.29;    2.05;    5.18;    2.93;    5.37;    6.13;
   5.31;    0.50;    2.07;    2.56;    3.28;    0.67;    5.94;   -1.00;    0.04;   -1.00;
  73.81;    1.54;    0.79;    0.84;    0.23;   -1.00;   26.00;
```

FAYCLO3COAPU

```
begin      integer  t, F1, F2, w, n, x, y, z;
                find (100, [PEARSON1] ); open (20); open (30);
                interchange (100);
                t := read (20); n := read (20);
       comment t = number of O.T.U.'s.  n = number of characters;
         begin real array  stock[1 : n]  ;
                   F1 := format ( [ss-ndd.dd;] ) ;
                   F2 := format ( [ss-ndd.dd:c] ) ;
                for x := 1 step 1 until t do
                begin      x := o:
                      for    y := 1 step 1 until n do
                      begin stock  y := read (20);
                         if  z > 8 then begin write (30,F2,stock[ y ] );
                              z := o; end
                         else begin write (30,F2,stock [ y]  );
                                      z := z + 1;
                               end;
                      end;  write binary (100, stock, [ modify] );
                end;
         end;      close (20); close (30); close (100);
end;
```

```
→ESTABLISH FAYCL0400APU;STANDARDISED CHARACTER STATES AND CORRELATION
 COEFFICIENTS AND TAXONOMIC DISTANCE  PEAR4;0/PL;→
begin library A0,A1,A5,A7,A8,A9,A15;
    integer t,n,qr; open(20); t:=read(20); n:=read(20); qr:=t;
begin  integer x,y,sumnc,z,W1,W2,W3,W4;
    real sum1,t2,sum2,n1,t1,mean,meansq,sd,scs,item1,item2,item,sum6,
         sum7,sum3,sum4,sum5,nom,denom,numer2,numer1,coeff,dist;
      real array cu[1:n,1:t],store[1:528],stove[1:n],stash[1:1056];
      open(30); find(100,[PEARSON1]);
         comment t=number of otus. n=number of characters;
     for y:= 1 step 1 until t do
     begin read binary(100,stove,[modify]);
          for x:= 1 step 1 until n do
          begin cu[x,y]:= stove[x]; end;
     end;
        for n1:=1 step 1 until n do
        begin sumnc:=0; sum2:=sum1:=0;
               for t1:=1step 1 until t do
                begin item:=cu[n1,t1];if item<0 then
                        sumnc:=sumnc+1 else
                      begin if item=0 then item:= 0.00001; sum1:=sum1+item;
                           sum2:=sum2+item↑2; end;
                 end; if t-sumnc=0 then begin  t:=2; sumnc:=1; end;
         sd:=sqrt((sum2-meansq)/x);
           if t=2 then t:=qr;
          for t1:=1 step 1 until t do
          begin item:=cu[n1,t1]; if sd=0 then sd:=0.00001;
                 scs:=((item-mean)/sd)+5;
                if item<0 then cu[n1,t1]:=item else
                cu[n1,t1]:=scs;
          end;
          end; z:=0;INTERCHANGEinterchange(100);

          W1:=format([≠d.nddddsssssss]);
          W2:=format([d.nddddsssssss]);
          W3:=format([dddsssssss]);
          W4:=format([dddc]);
          write text(30,[[3c]CORRELATION[2s]DISTANCE[3s]IDENTIFIERS[5c]]);
         for t1:=1 step 1 until t do
     begin    for t2:=t1 step 1 until t do
          begin  sumnc:=0; n1:=0;  sum6:=sum7:=sum3:=sum4:=sum5:=0;
                for n1 := 1 step1 until n do
                     begin item1:=cu[n1,t1];  item2:=cu[n1,t2];
                           if item1×item2<2 then sumnc:=sumnc+1  else
                              begin sum6:=sum6+item1; sum7:=sum7+item1↑2;
                                     sum3:=sum3+item2; sum4:=sum4+item2↑2;
                                      sum5:=sum5+(item2×item1);
                          end;
               end;
            nom:=n-sumnc; denom:=sum5-((sum6×sum3)/nom);
              numer2:=sum4-(sum3↑2/nom);
            numer1:=sum7-(sum6↑2/nom);  coeff:=denom/sqrt(numer1×numer2)
            dist:=sqrt((sum7+sum4-2×sum5)/nom);
            write(30,W1,coeff);
            write(30,W2,dist);
            write(30,W3,t1);
            write(30,W4,t2);
            if t2=t and t1=t then begin   ifz=528 then goto PETAL;
                                    for x:=1+z step 1 until 528 do
                                   begin store[x]:=-.999; stash[x×2-1]:=-1;
                                          stash[x×2]:=0;
                                   end;
PETAL:                             write binary(100,store,[store]);
                                  write binary(100, stash,[stash]);
                           end
else begin if t1=t2 then goto CARPEL;
       if z<528 then begin z:=z+1; store[z]:=coeff; stash[z×2-1]:=t1;
                                  stash[z×2]:=t2;
                         end
                  else begin z:=0;write binary(100,store,[store]);
                                write binary(100,stash,[stash]);
                              z:=z+1; store[z]:=coeff; stash[z×2-1]:=t1;
                             stash[z×2]:=t2;
                         end;
      end; CARPEL:    end; end; close(30); close(100);
end; close(20);
end→
```

FAYCL04.

FAYCL0404KPU 20/12/66
STR3C

CORRELATION	DISTANCE	IDENTIFIERS	
+1.0000	0.0000	001	001
+0.3487	0.7807	001	002
-0.0570	0.9719	001	003
+0.3027	0.8042	001	004
-0.2187	1.0154	001	005
-0.1967	1.1272	001	006
+0.0640	1.1039	001	007
+0.0935	0.9248	001	008
-0.0427	0.9440	001	009
+0.1303	0.8569	001	010
-0.3074	1.0665	001	011
-0.2257	1.1481	001	012
-0.1257	0.9873	001	013
-0.0636	0.9577	001	014
+0.2323	0.8392	001	015
-0.1465	0.9561	001	016
-0.1404	1.3762	001	017
-0.0847	0.9477	001	018
+0.1831	0.8405	001	019
+0.0074	0.9139	001	020
+0.1149	0.9211	001	021
+0.1044	0.8813	001	022
-0.0561	1.1491	001	023

```
FAYCL0800APU→
begin library A1,A5,A7,A12,A8;
       integer n,t,q,qr; open(20); n:=read(20);t:=read(20);q:=read(20);
qr:=read(20); comment n=number of characters. t=number of O.TU.S. q=number of
             O.T.U.S in group1. qr=number of O.T.U.S in group2
             This Program is essentially the same as FAYCL06 Bbut misses
             out up to 10 characters by reading in the character numbers
             into X. If <10 characters are to be missed out values of 200
              are read in;
     begin integer F1,x,y,cat,X1,X2,X3,X4,X5,X6,X7,X8,X9,X10;
          real dog;
         real array store[1:n,1:t],stash[1:n];
         integer array tich[1:q],small[1:qr];
        open(10); open(11); find(100,[PEARSON1]);
         X1:=read(20);X2:=read(20); X3:=read(20); X4:=read(20);X5:=read(20);
         X6:=read(20); X7:=read(20); X8:=read(20); X9:=read(20);
         X10:=read(20);
         F1:=format([-nddd.dddd;]);
         for x:=1 step 1 until t do
         begin read binary(100,stash,[modify]);
              for y:=1 step 1 until n do
              begin if stash[y]=-1 then stash[y]:=0; store[y,x]:=stash[y];
              end;
        end;
        for x:= 1 step 1 until q do
        begin tich[x]:=read(20); end;
        for x:=1 step 1 until qr do
        begin small[x]:=read(20); end;
       for x:=1 step 1 until q do
       begin for y:= 1 step 1 until n do
             begin if y=X1 then goto FRED;
                   if y=X2 then goto FRED;
                   if y=X3 then goto FRED;
                   if y=X4 then goto FRED;
                   if y=X5 then goto FRED;
                   if y=X6 then goto FRED;
                  if y=X7 then goto FRED;
                  if y=X8 then goto FRED;
                 if y=X9 then goto FRED;
                  if y=X10 then goto FRED;
                     cat:=tich[x]; dog:=store[y,cat]; write(10,F1,dog);
FRED:             end;
          gap(10,30);
      end;
      for x:=1 step 1 until qr do
      begin for y:=1 step 1 until n do
            begin if y=X1 then goto BLOGS;
                  if y=X2 then goto BLOGS;
                  if y=X3 then goto BLOGS;
                   ify=X4 then goto BLOGS;
                  if y=X5 then goto BLOGS;
                  if y=X6 then goto BLOGS;
                  if y=X7 then goto BLOGS;
                  if y=X8 then goto BLOGS;
                  if y= X9 then goto BLOGS;
                  if y=X10 then goto BLOGS;
                  cat:=small[x]; dog:=store[y,cat]; write(11,F1,dog);
BLOGS:          end;
      gap(11,30); end;
   end; close(20); close(10); close(11); close(100);
end→
```


FAYCL08.

FAYCLOS01KPU 23/12/66

[illegible]IENT	IDENTIFIERS		CLUSTER JOINED
+0.9328	034	035	300
+0.8416	005	006	301
+0.8343	025	048	302
+0.8080	020	022	303
+0.8046	028	049	304
+0.8027	047	058	305
+0.7622	008	010	306
+0.7613	043	046	307
+0.7459	037	038	308

FAYCLO5.


```
→ESTABLISH FAYCLO500APU;
WEIGHTED PAIR CLUSTER ANALYSIS;
O/PL;→begin   library A0,A4,A1,A5,A7,A8,A9,A15;
    integer a,n,q,r,z,c,e,F1,F2,F3,F4,y,x,nit1,f,g,nit,nerk;
    real nig;
    integer array tich[1:20],place[1:400];
    real procedure cosh(x); real x;
           begin real z; z:=exp(x); cosh:= 0.5×(z+1.0/z); end;
    procedure fishz(trans); real trans;
           begin trans:= (ln(1+trans)-ln(1-trans))/2; end;
    procedure defish(trans); real trans;
           begin real y7; y7:=cosh(trans);
                trans:=(if trans<0 then -1 else 1)×sqrt((y7↑2-1)/y7↑2) ;end;
  procedure stuff(frig,frog); integer frig,frog;
         begin integer frag; if frig> frog then begin frag:=frog; frog
                           :=frig; frig:=frag;end;
              frig:=frig×1000; frog:=frig+frog;
         end;
   procedure unstuff(frig,frag,frog); integer frig,frag,frog;
         begin integer frug; frog:=entier(frig/1000); frug:=frog×1000;
                frag:=frig-frug;
         end;
   procedure bunch(i1,g1,f1,sick,sock,plonk,b1,c1);
             integer c1,f1,g1,i1,b1;
         real array sick;
     integer array plonk,sock;
    begin integer n1,d1,l1,m1,a1,e1,y1,x1,l2,m2;
    real g2,g3;
     for x1 := 1 step 1 until 400 do
         begin plonk[x1]:= -1;
         end;    n1:=0;
         for a1:=b1+1 step 1 until c1 do
         begin d1:=sock[a1]; unstuff(d1,l1,m1);
               if l1=f1 or l1=g1 then goto LEAP;
               if m1=g1 or m1=f1  then goto LOOP else goto HOP;
LEAP:          l1:=i1; stuff(m1,l1); sock[a1]:=l1; n1:=n1+1;
               plonk[n1]:=a1; goto HOP;
LOOP:          m1:=i1; stuff(m1,l1); sock[a1]:=l1; n1:=n1+1; plonk[n1]:=a1;
HOP:     end;
         for x1:=1 step 1 until n1+1 do
         begin d1:=plonk[x1];  if d1<0 then goto ANTHER;
               if d1<1 then goto STAMEN;

               m2:=sock[d1]; unstuff(m2,l1,m1);
               for y1:=x1+1 step 1 until n1 do
               begin e1:=plonk[y1]; if e1<1 then goto LIGULE;
                   a1:=sock[e1]; unstuff(a1,l2,m2); if l1=l2 and m1=m2
                  then begin g2:=sick[d1]; g3:=sick[e1]; fishz(g2);
                  fishz(g3); g2:=(g2+g3)/2; defish(g2); sick[d1]:=g2;
                  sick[e1]:=-0.990; sock[e1]:=0; plonk[x1]:=0;
                 plonk[y1]:=0;
                  end else goto LIGULE;  goto STAMEN;
LIGULE:             end;
STAMEN      : end;
ANTHER:      x1:=0; for a1:=b1+1 step 1 until c1-1 do
                begin if sick[a1+1]>sick[a1] then begin g2:=sick[a1];
                      sick[a1]:=sick[a1+1]; sick[a1+1]:=g2; m2:=sock[a1];
                      sock[a1]:=sock[a1+1]; sock[a1+1]:=m2; x1:=x1+1; end;
              end;
               if x1>0 then goto ANTHER; c1:= c1-entier(n1/2);
   end;
   open(20); open(30); find(100,[PEARSON1]); find(101,[********]);
   a:=read(20); n:=read(20);
  comment a=number of coefficients. n = number of arrays;
  q:=1056; r:=528; z:=0; c:=0; e:=0;
  F1:= format([s+d.ddddssssssssssssss]);
  F2:= format([ dddssssssss]);
  F3:= format([ dddssssssssssssss]);
  F4:= format([dddccc]);
  write text(30,[COEFFICIENT[12s]IDENTIFIERS[12s]CLUSTER*JOINED[10c]]);
  begin integer qr,qv,qw,qz;
        integer array stash[1:q], bigtwo[1:a];
         real array conv[1:q], store[1:r], bigone[1:a], stoke[1:q];
         if a<529 then qr:=a else begin qv:=entier(a/528); qw:=a-qv×528;
          qr:=528; qz:=0; end;
         for x:= 1 step 1 until n do
         begin read binary(100,store,[store]);
               read binary(100,conv,[stash]);
               for nit:= 1 step 1 until q do
               begin stash[nit]:=conv[nit]; end;
               for y:= 1 step 1 until qr do
               begin z:=z+1; bigone[z]:= store[y]; nit:=stash[y×2-1];
                     nerk:=stash[y×2]; stuff(nit,nerk); bigtwo[z]:=nerk;
             end; if a>528 then begin qz:=qz+1; if qz=qv then qr:=qw; end;
          end;
          for nig:= +.90 step -.10 until -1.00 do
          begin z:=0; for x:= 1 step 1 until a do
               begin if bigone[x]> nig then begin z:=z+1; stoke[z]:=bigone[x
                        stash[z]:= bigtwo[x]; bigone[x]:=-2.0;
                                            end;
               end;
               if z>0 then begin write binary(101,stoke,[stoke]); c:= c+1;
                for nit:=1 step 1 until z do
               begin conv[nit]:=stash[nit]; end;
                               tich[c]:=z; write binary(101,conv,[stack]);
                         end;
          end;
           interchange(101);
         for x:= 1 step 1 until c do
    begin z:= tich[x]; read binary(101,stoke,[stoke]);
          read binary(101,conv,[stack]);
          for nit:=1step 1 until z do
          begin tash[nit]:=conv[nit]; end;
JERK:     n:=0; for y:= 1 step 1 until z-1 do
          begin if stoke[y+1]>stoke[y] then
                begin nig:=stoke[y]; stoke[y]:=stoke[y+1]; stoke[y+1]:=nig;
                      nit1:=stash[y]; stash[y]:=stash[y+1]; stash[y+1]:=nit1;
                   n:=n+1;
                end;
         end;
         if n>0 then goto JERK else
          begin for y:= 1 step 1 until z do
                begin bigone[e+y]:=stoke[y]; bigtwo[e+y]:=stash[y];
                end;
                e:=e+z;
          end;
    end;

    n:=300; for x:=1 step 1 until a do
    begin nig:=bigone[x]; nit1:=bigtwo[x]; unstuff(nit1,f,g);
          comment the larger identifier is put into f and the smaller
                 into g;
          if nig<-0.9899 then goto Thank God for the end;
          if f<299 then begin z:=n; n:=n+1; write(30,F1,nig);
                               write(30,F2,g); write(30,F3,f);
                               write(30,F4,z);
                              bunch(z,g,f,bigone,bigtwo,place,x,a);
                        end
          else begin if f>299 and g<299 then z:=f else z:=g;
      write(30,F1,nig); write(30,F2,g); write(30,F3,f);
                     write(30,F4,z); bunch(z,g,f,bigone,bigtwo,place,x,a);
               end;
      end;
  Thank God for the end:
    end;  close(20); close(30); close(100); close(101);
  end→
```

FAYCLO5.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allioni (1755) Flora Pedemontana

Alston, R.E. & Turner, B.L. (1959) 'Application of paper chromatography to systematics: Recombination of parental biochemical components in a Baptisia hybrid population' Nature, 184, 285-6.

Alston, R.E. & Turner, B.L. (1963) 'Natural hybridisation among species of Baptisia (Leguminosae)'. American Journal of Botany, 50, 159-173.

Alston, R.E. et al. (1965) 'Hybrid compounds in natural interspecific hybrids' Proceeding of the National Academy of Science, 54, no. 5, 1458.

Anderson, E. (1953) 'Introgressive hybridisation' Biological Review 28 280-307.

Anderson, E. (1954) 'Plants, Man and Life' London.

Anderson, E & Whitaker, T.W. (1936) 'A quantitative comparison of specific and generic differences in Betulaceae' Journal of the Arnold Arboretum 15, 43-49.

Anderson, E & Whitaker, T.W. (1936) 'Speciation in Uvularia' Journal of the Arnold Arboretum, 15, 28-42.

Avers, C.J. (1954) 'Chromosome behaviour in fertile triploid Aster hybrids' Genetics, 39, no. 1. 117-126.

Babcock, E.B. (1947) 'The Genus Crepis. Part One: The taxonomy, phylogeny and evolution of Crepis' University of California Publication in Botany, 21.

Baker, H.G. (1961) 'Rapid speciation in relation to changes in the breeding system of plants' Recent Advances in Botany, 881-5.

Baksay, L. (1956) 'Cytotaxonomical studies on the flora of Hungary' Annales Historico-Naturales Musei Nationalis Hungarici, VII, 320.

Baksay, L. (1957) 'The cytotaxonomy of the species Chrysanthemum maximum Ram, Centaurea montana L., Serratula lycopifolia.' Annales Historico-Naturales Musei Nationalis Hungarici, VIII 155-168.

Baldwin, J.T., Jr. (1941) 'Galax: the genus and its chromosomes' Journal of Heredity, 32, 249-254.

Basak, S.L. & Jain, H.K. (1963) 'Autonomous and interrelated formation of chiasmata in Delphinium chromosomes' Chromosoma 13, 577-587.

Bate-Smith, E.C. (1959) 'A chemical approach to plant taxonomy' Low Temperature Research Station Record Memorandum No. 344. Cambridge.

Bate-Smith, E.C. (1961) 'Chromatography and taxonomy in the Rosaceae, with special reference to Potentilla and Prunus' Journal of the Linnean Society (Botany) 88, 39-54.

Bate-Smith, E.C. (1962) 'The phenolic constituents of plants and their taxonomic significance' Journal of the Linnean Society (Botany) 58, 95-173.

Beutler, E. (1963) 'Autosomal inactivation' Lancet 1, 1242.

Block, Durrum, Zweig, (1958) 'Paper chromatography and paper electrophoresis' Academic Press, New York.

Böcher, T & Larsen, K. (1957) 'Cytotaxonomical studies in the Chrysanthemum leucanthemum complex' Watsonia 4, pt. 1, 11-16.

Brandt, Jean-Pierre, (1961) 'Cytotaxinomie et cytogéographie de Veronica prostrata L.' Bullétin de la Société Neuchateloise des Sciences naturelles. 84, 37-88.

Braun-Blanquet, J. (1948) 'La Végétation Alpine des Pyrénées Orientales'

Briquet and Cavillier (1916) (Burnat) Flores des Alpes Maritimes, 6.

Brown, S.W. & Zohary, D. (1955) 'The relationship of chiasmata and crossing over in Lilium formosanum' Genetics 40, 850-873.

Cain, A.J. & Harrison, G.A. (1958) 'An analysis of the taxonomist's judgement of affinity' Proceedings of the Zoological Society of London, 131, Pt. 1, 85-98.

Cain, A.J. & Harrison, G.A. (1960) 'Phyletic Weighting' Proceedings of the Zoological Society of London, 135, Pt. 1, 1-31.

Camp, W. & Gilly, C.L. (1943) 'The structure and origin of species' Brittonia, 4, 232-358.

Celarier, R.P.. & Mehra, J.L. (1958) 'Determination of Polyploidy from Herbarium specimens' Rhodora, 60, 89-97.

Clapham, Tutin and Warburgh (1962) 'Flora of the British Isles'.

Clausen, J, Keck, D.D. & Hiesey, W.M., (1939) 'The concept of the species based on experiment' American Journal of Botany, 26, 103-106.

Clausen, J., Keck, D.D. & Hiesey, W.M., (1945) 'Experimental studies on the nature of the species II. Plant evolution through an autopolyploidy, with examples from the Madiinae' Carnegie Institute of Washington Publication, 564,.

Coombe, D.E. & Frost, L.C. (1955) 'The Heaths of the Cornish Serpentine'. Journal of Ecology, 44, No. 1, 226.

Cooper, D. & Mahoney, K.L. (1935) 'Cytological observations on certain compositae' The American Journal of Botany 22, 843-848.

Darlington, C.D. (1937) Cytology Pt. 1. London : Churchill.

Darlington, C.D. (1939) 'The Evolution of genetic systems' London : Cambridge University Press.

Darlington, C.D. & Mather, K. (1932) 'The origin and behaviour of chiasmata III. Triploid Tulipa' Cytologia, 4, 1-15.

Davies, D.R. (1958) 'Male parthenogenesis in barley' Heredity, 12, 493-498.

Davis, P.H. & Heywood, V.H., (1963) 'Principles of angiosperm taxonomy'.

Dawson, C.D.R. (1941) 'Tetrasomic inheritance in Lotus corniculatus' Journal of Genetics, 42, 41.

De Candolle, (1837) Prodromus VI, 46-47.

Dowrick, G.J. (1952) 'The Chromosomes of Chrysanthemum I: The species' Heredity, 6, 365-375.

Dowrick, G.J. (1953) 'The chromosomes of Chrysanthemum III. Meiosis in Chrysanthemum atratum' Heredity, 7, 219.

Duckert, M. & Favarger, C. (1956) 'Sur l'éxistence dans le Jura d'une forme diploide de Chrysanthemum leucanthemum L.' Bûlletin de la Société botanique Suisse. 66, 134-146.

Dulac, L'Abbé, J. (1867) Flore du Department des Hautes-Pyrénées.

Durrant, A. (1960) 'Expected frequencies of chromosome associations in tetraploids with random chiasma formation" Genetics, 45, 779-783.

Elliott, C.G., (1958) 'Environmental effects on the distribution of chiasmata among nuclei and bivalents and the correlation between bivalents" Hereditary, 12, 429-439.

Favarger, C. (1959) 'Distribution en Suisse des race chromosomiques de Chrysanthemum leucanthemum L.' Bûlletin de la Société botanique Suisse. 69, 26-46.

Favarger, C. (1963) 'Sur la présence de chromosomes B dans l'éspece collective Chrysanthemum leucanthemum L.' Bûlletin de la Société Neuchateloise des Sciences Naturelles, 86, 100.

Favarger, C & Villard, M. (1965) 'Contribution á la cytotaxinomie et á la cytogéographie des marguerites d'Europe: Chrysanthemum leucanthemum L. et taxa voisins'. Compte Rêndus Academié de Science, Paris, 261, 497-498.

Favarger, C & Villard, M. (1966) 'Nouvelles recherches cytotaxinomiques sur Chrysanthemum leucanthemum L. sensu lato' Bûlletin de la Société botanique Suisse.' 75, 57-78.

Fernald, M.L. (1903) 'Chrysanthemum leucanthemum and the American White weed' Rhodora, 5, 177-181.

Fernald, M.L. (1950) 'Gray's Manual of Botany'.

Fitzgerald, P.N., (1965) 'Differential contraction of large and small chromosomes in cultured leucocytes of man' Cytogenetics, 4, 65.

Ford, E.B. (1953) 'The genetics of polymorphism in the Lepidoptera' Advances in Genetics, 5, 43-87.

Garber, E.D. (1955) 'Cytogenetics of sorghum 1. The orientation of interchange complexes and quadrivalents at metaphase 1 in Sorghum purpureosiriceum' Botanical Gazette, 116, 369-372.

Gilmour, J.S.L. & Gregor, J.W. (1939) 'Demes: a suggested new terminology' Nature, 144, 333-334.

Gilmour, J. (1940) 'Taxonomy and Philosophy' The New Systematics, 461-474. Ed. J.S. Huxley.

Gilmour, J.S. & Heslop-Harrison, J. (1954) 'The deme terminology and the units of micro evolutionary change' Genetica 27, 147-1611

Gregor, J.W. (1939) 'Experimental Taxonomy IV. Population differentiation in North American and European sea plantains allied to *Plantago maritima* L. New Phytologist, 38, 293.

Hall, B.M. (1955) 'Binomial analysis of chromosome behaviour in hybrid plants' Proceedings of the Pennsylvanian Academy of Science' 29, 121-126.

Hamilton, M. (1965) 'Computer Programmes for the medical man - a solution' British Medican Journal, 2, 1048-1050.

Harberd, D.J. & Watson, P.J., (1962) 'A note on segregation ratios in tetraploid grasses' Scottish Plant Breeding Station Record.

Harling, G. (1928) 'Embryological studies in the Compositae. Pt. 1. Anthemideae-Chrysantheminae. Acta Horti Bergiani, 16, 1-56.

Hegi, G. (1928) 'Illustrierte Flora von Mitteleuropa' Hausen Verlag München, 6, 590 and seq.

Heslop-Harrison, J. (1953) 'New Concepts in Flowering Plant Taxonomy' London.

Heslop-Harrison, J. (1962) 'Purposes and procedures in the taxonomic treatment of higher organisms' Microbial Classification (Ed. Ainsworth, G.C. & Sneath, P.H.A.) 14-36.

Hewitt, D. (1964) 'Population cytology of British grasshoppers 1'. Chromosoma, 15, 211-230.

Heywood, V.H. (1958) 'The Presentation of Taxonomic Information' Leicester University Press.

Heywood, V.H. (1959) 'A check list of the Portuguese Compositae - Chrysantheminae'. Agronomia lusitana, 20, 205-216.

Heywood, V.H. (1960) 'The taxonomy of polyploids in Flora Europea' Feddes Repertorium specierum novarum regni vegetablis.' 63, 179-192.

Heywood, V.H. (1963) 'The 'Species aggregate' in theory and practice' Regnum Vegetabile 27, 26-37.

Heywood, V.H. & McNeill, J. Editors. (1964) 'Proceedings of the Symposium "Phyletic and Phylogenetic" classifications' Systematics Association Publication, 6.

Hilpert, G. (1957) 'Effect of selection for meiotic behaviour in autotetraploid rye.' Hereditas, 43, 318-322.

Horvatic, S. (1935) 'Neuer Beitraz zur Kenntnis der Leucanthemum Formen in der Flora Jugoslaviens'. Acta botanica Instituti botanici Universitatis zagrebiensis, 10, 61-100.

Huxley, J.S. (1942) 'Evolution: the modern Synthesis' London.

Jain, H.K. (1957) 'Effect of high temperature on meiosis in Lolium: nucleolar inactivation' Heredity, 11, 23-36.

John, B & Henderson, S.A. (1962) 'Asynapsis and polyploidy in Schistocerca paranensis' Chromosoma, 13, 111-147.

Jones, K. (1954) 'A consideration of certain aspects of cytotaxonomic investigation in the light of results obtained in Holcus.' Report of the 8th International Botanical Congress, Paris, 9 and 10, 75.

Jones, K. (1956) 'Species differentiation in Agrostis 1. Cytological relationships in Agrostis canina L.' Journal of Genetics, 54, 370-376.

Jones, K. (1956) 'Species differentiation in Agrostis III. Agrostis gigantea Roth. and its hybrids' Journal of Genetics, 54, no.3. 394.

Jones, K. (1958) 'Cytotaxonomic studies in Holcus. 1. The chromosome complex in Holcus mollis L.' New Phytologist, 57, 191-210.

Jones, K. & Borill, M. (1962) 'Chromosome status, gene exchange and evolution in Dactylis. 111. The role of interploid hybrids.' Genetica, 32, 296-322.

Jones, K. (1964) 'Chromosomes and the nature and origin of Anthoxanthum odoratum L.' Chromosoma, 15, 248-274.

Jones, R.E. & Bamford, R. (1942) 'Chromosome number in the progeny of triploid Gladiolus with special reference to the contribution of the triploid' The American Journal of Botany, 29, 807-813.

Kendrick, W.B. & Proctor, J. (1964) 'Computer taxonomy in the Fungi Imperfecti'. Canadian Journal of Botany, 42, 65-88.

Klinger, H.P. et al. (1965) 'D.N.A. content and area of sex chromatin in subjects with structural and numerical aberrations of the X chromosome'. Cytogenetics, 4, 96-116.

Klundstedt, H. (1937) 'On some tetraploid spermatocytes in Chrysochraon dispar (Orth.)' Memoranda Societatis pro fauna et flora fennica. 12, 194-209.

Lambert, J.M. & Dale, M.B., (1964) 'The use of statistics in phyto-sociology' Advances in Ecological Research, 2.

Lamm, R., (1936) 'Cytological studies in inbred rye.' Hereditas (Lund) 22, 217-240.

Lederbour, (1844) 'Flora Rossica' 2.

Lewis, K.R. & John, B. (1963) 'Chromosome Marker' J. & A. Churchill Ltd. London.

Linneas, C. (1737) 'Hortus Cliffortianus'.

Linneas, C. (1753) 'Species Plantarum'.

Long, C.A. (1966) 'Dependency in Taxonomy' Taxon, XV. no. 2, 49.

Löve A. & Löve, D., (1956) 'Cytotaxonomical conspectus of the Icelandic Flora'. Acta Horti Gotoburgensis, 24, 4.

Lubischen, A.A., (1963) 'On some contradictions in general taxonomy and evolution'. Evolution, 17, 414-430.

Martin, W.R. & Smith, F.H., (1955) 'Megagametophyte development in Chrysanthemum leucanthemum'. Botanical Gazette, CXVI, 243.

Masima, I. 'Meiosis in diploid and tetraploid plants of Linum angustifolium buds'. Cytologia, 14, 55-69.

Mather, K. (1935) 'Chromosome behaviour in a triploid wheat hybrid'. Zeitschrift für Zellforschung und mikroskopische Anatomie. 23, 117-138.

Mather, K. (1936) 'Competition between bivalents during chiasma formation'. Proceedings of the Royal Society, B120, 208-227.

McCollum, G.D., (1958) 'Comparative studies of chromosome pairing in natural and induced tetraploid Dactylis'. Chromosoma, 9, 571.

Michener, C.D. & Sokal, R.R., (1958) 'A quantitative approach to a problem in classification'. Evolution, 11, 130-160.

Moore, R.C., (1965) 'A Biometric analysis of the chromosomes of the Marsupials - Macropus major, Macropus rufus and Potorous tridactylis'. Cytogenetics, 4, 145-156.

Moore, R.C. & Gregory, G. (1963) 'Biometrics of the karyotype of Proteon nodon bicolor with reference to the limitation in accuracy of identifying human chromosomes'. Nature, 200, 234.

Morishima, H. & Oka, H., (1960) 'The pattern of interspecific variation in the genus Oryza: its quantitative representation by statistical methods'. Evolution, 14, 153-165.

Morrison, J.W. (1956) 'Chromosome behaviour and fertility of tetra Petkus rye' Canadian Journal of Agricultural Science, 36, 157-165.

Morrison & Rajhathy, (1960) 'Frequency of quadrivalents in auto tetraploid plants'. Nature, 187, 529.

Mulligan, G.A. (1958) 'Chromosome races in the Chrysanthemum leucanthemum complex'. Rhodora, 60, 122-125.

Mulligan, G.A. (1960) 'Polyploidy in Canadian Weeds' Canadian Journal of Genetics and Cytology, 2, 150-161.

Müntzing, A. (1958) 'A new category of chromosomes' Proceedings of the Tenth International Congress of Genetics, 1, 453-467.

Myers, W.M. & Hill, H.D., (1940) 'Studies of chromosome association and behaviour and occurrence of autotetraploid grass species, orchard grass, tall oat grass and crested wheat grass'. Botanical Gazette, 102, 236-255.

Negodi, G., (1937) 'Nuovi reperti cariologici su Faneroganic' Atti della Società dei naturalisti e mathematici, 68, 9-11.

Nybom, N. (1964) 'Thin-layer chromatographic analysis of Anthocyanidins' Physiologia Plantarum, 17, 157-164.

Oksala, T. (1952) 'Chiasma formation and chiasma interference in the Odonata' Hereditas, 38, 459.

O'Mara, J. G. (1948) 'Acetic acid methods for chromosome studies at prophase and metaphase in meristems' Stain Technology, 23, 201-4.

Owen, A.R.G. (1949) 'A possible interpretation of the apparent interference across the centromere found by Callan and Montalenti in Culex pipiens' Heredity, 3, 357-367.

Orth, (1926) in Tischler, G. (1950) 'Die Chromosomenzahlen der Gefässplanzen Mitteleuropas'

Palmer & Funderburk, (1965) 'Secondary constrictions in human chromosomes' Cytogenetics, 4, 261-267.

Patau, K. (1960) 'The identification of individual chromosomes, especially in man' American Journal of Human Genetics, 12, 250-276.

Patau, K. (1964) 'Identification of chromosomes' Human Chromosome Methodology, Ed. Yunis, J.J. 155.

Pearson, K. & Yule, G.U., (1902) 'Variation in ray flowers of Chrysanthemum leucanthemum' Biometricka.

Polya, L. (1950) 'Magyaroszagi noven fajok bromoszomaszamai II' Annales biologicae Universitatis debreceniensis 1, 46-56.

Povilaitis, B. & Boyes, J.W., (1956) 'A cytological study of autotetraploid red clover' American Journal of Botany, 43, 169-174.

Proctor, J.R. & Kendrick, W.B. (1963) 'Unequal weighting in Numerical Taxonomy' Nature, 197, 716-717.

Randerath, K. (1963) 'Thin-layer Chromatography' Verlag Chemie Academic Press.

Rees, H. (1958) 'Differential behaviour of chromosomes in Scilla'. Chromosoma, 9, 185-192.

Rees, H. (1961) 'Genotypic control of chromosome form and behaviour' Botanical Review, 27, 288-318.

Riley, R., Chapman, V & Kimber, G., (1959) 'Genetic control of chromosomes pairing in intergeneric hybrids with wheat' Nature, 183, 1244-46.

Rohweder, (1937) 'Versuch zur Erfassung der mengemässigen. Bedeckung des Darss und Zingst etc.,' Planta, 27,.

Rouy, (1905) 'Flore de France' Paris, IX.

Rowlands, D.G. (1958) 'The control of chiasma frecuency in Vicia faba' Chromosoma 9, 176-184.

Ruddle, E.H. (1964) "Quantivation and automation of chromosomal data with special reference to the chromosomes of the Hampshire pig' Cytogenetics of cells in culture. Ed. Harris, J. 3, 273.

Sarker, P.K., Bidwell, O.W., Marens, L.F., (1966) 'Selection of characteristics for numerical classification of soils' Proceedings of the American Soil Science Society, 30, no. 2, 239-279.

Satina, S. & Blakeslee, A.F., (1937) 'Chromosomes behaviour in triploids in Datura stramonium 1. The male gametophyte' American Journal of Botany, 24, 518-527.

Sharma, A.K., & Sharma, A. (1965) 'Chromosome Techniques, Theory and Practice'. London : Butterworth.

Shaw, M.W., & Krooth, R.S. (1964) 'The chromosomes of the Tasmanian rat kangeroo (Potorons tridactylis apicalis)' Cytogenetics, 3, 19-33.

Shimotomai, N. (1938) 'Chromosomenzahlen bei einige Arten von Chrysanthemum' Zeitschrift für induktive Abstammunge-Vererbungslehre.

Skalinska, M. et al. (1961) 'Further additions to chromosome numbers of Polish Angiosperms' Acta Societatis Botanicorum Poloniae, 30, No. 3.

Skalinska, M. (1963) 'Cytological studies in the flora of the Tatra Mountains: A synthetic review' Acta Biological Cracoviensis Series: Botanica 6.

Skalinska, M. et al. (1964) 'Additions to chromosome numbers of Polish Angiosperms (Fifth contribution)' Acta societatis botanicorum poloniae. 38, No. 1.

Sneath, P.N.A., & Sokal, R.R., (1962) 'Numerical taxonomy' Nature, 193, 855-860.

Sokal, R. & Rohlf, F.J., (1962) 'The comparison of dendograms by objective methods' Taxon. 11, No. 2, 39.

Sokal, R.R., & Sneath, P.N.A., (1963) 'Principles of Numerical Taxonomy' Academic Press.

Solberg, O.T. (1964) 'Infraspecific variation in the Gutierrezia sarothrae complex (Compositae-Astereae)' Contributions from the Gray Herbarium of Harvard University, p. 67.

Stebbins, G.L. (1947) 'Types of polyploids: their classification and significance' Advances in Genetics. 1, 403-429.

Stebbins, G.L. (1950) 'Variation and Evolution in Plants' Columbia University Press.

Stebbins, G. L.,(1956) 'Cytogenetics and evolution of the grass family' American Journal of Botany. 43, 890-905.

Stebbins, G.L., (1957) 'Self fertilisation and population variability in the higher plants' American Naturalist, 91, 337.

Stebbins, G.L., (1958) 'Longevity, habitat, and release of genetic variability in the higher plants' Cold Spring Harbour Symposia on Quantitative Biology. 23, 365-377.

Steele, B., (1955) 'Soil pH and base status as factors in the distribution of calcicoles' Journal of Ecology, 43, No. 1, 120-132.

Stephens, S.G., (1961) 'Recombination between supposedly homologous chromosomes of Gossypium barbadense L. and G. hirsutum'. Genetics, 46, 1483-1500.

Stone, L.H.A., & Mather, K. (1932) 'The origin and behaviour of chiasmata IV. Diploid and triploid Hyacinthus' Cytologia, 4, 16-25.

Taft, P.D. et al. (1965) 'Sex chromatin body size and its relation to X chromosome structure' Cytogenetics, 4, 87-95.

Tahara, M., (1915) 'Cytological studies on Chrysanthemum' Tokyo Botanical Magazine, 29, 48-50.

Tahara, M. (1921) 'Cytologische studien an einigen Kompositen' Journal of the College of Engineering, Imperial University of Tokyo. 43, no. 7.

Tansley, A.G., (1939) 'The British Islands and their vegetation' Cambridge University Press.

Thompson, J.B., (1956) 'Genotypic control of chromosome behaviour in rye II. Disjunction at meiosis in interchange heterozygotes' Heredity, 10, 99-108.

Torres, A.M., (1965) 'The chromosome races of Zinnia juniperifolia' The American Journal of Botany, 52, 760.

Tsuchiya, T., (1957) 'Fertility of autotetraploids and their hybrids in barley II Meiosis and fertility in tetraploid hybrids'. Seiken Ziho, 8, 27-32.

Turesson, G., (1929) 'Zur natur und Begrenzung der Arteneinheiten' Hereditas, 12, 323-334.

Valentine, D.H., (1949) 'The units of experimental taxonomy' Acta Biotheoretica, 9, 75-88.

Valentine, D.H., (1961) Presidential Address to the Yorkshire Naturalist's Union, Ilkley, December 3rd, 1960. The Naturalist, p. 3.

Valentine, D.H., & Löve, A., (1958) 'Taxonomic and Biosystematic categories' Brittonia, 10, no.4, 153-165.

Walters, S.M., (1962) 'Generic and specific concepts and the European Flora' Preslia, 34, 207-226.

Webb, D.A., & Hart, A.V., (1945) 'Contributions towards an understanding of the calcicole and calcifuge habit in some Irish plants' Scientific Proceedings of the Royal Dublin Society, 24, no.5. 19-28.

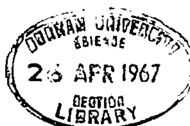
Whitehead, F.H., (1954) 'An example of Taxonomic Discrimination by Biometric methods' New Phytologist, 53, pt.3, 496-510.

Williams, W.T. et al. (1964) 'An objective method of weighting in similarity analysis' Nature, 201, 426.

Williams, W.T. & Lance, G.N., (1965) 'Logic of computer-based intrinsic classifications' Nature, 207, 159-161.

Williams, W.T. & Dale, M.B., (1965) 'Fundamental problems in numerical taxonomy' Advances in Botanical Research, 2.

Yanny-Wilson, J. (1959) 'Cytogenetics of Triploid Endymion non-scriptus (L) Garcke and E. hispanicus (Mill) Chouard' Cytologia, 23, no.4, 435-446.


26 APR 1967
LIBRARY