Cytogenetical studies in the genus primula

Eaton, R.D.

CYTOGENETICAL STUDIES IN THE GENUS PRIMULA

by

R.D. EATON, B.Sc. (University)

A thesis presented in candidature for the degree of Doctor of Philosophy of the University of Durham

1968

"Cytogenetical studies in the genus Primula"

Abstract of the Ph.D. thesis presented by R. D. Eaton, 1968.

1. Different classifications of the taxa described here have been produced by formal taxonomists. The degree of relationship of the taxa, and their levels of importance have varied. Experimental taxonomists have produced a different picture, of a closely knit group with well defined relationships.

2. Genetics and cytology are used in this work in an effort to clarify the situation.

3. The control of characters of importance in classification is shown to be similar to that found in other groups.

4. A possible association between morphological characters and fertility factors is described, and compared with systems described in the literature.

5. The validity of cytological methods of determining relationships is discussed.

6. Meiosis in a haploid plant is used to show the absence of autosyndesis, and hence to deduce that pairing in interspecific hybrids must indicate homologies between specific genomes.

7. The analysis of meiosis in interspecific hybrids is used to reveal relationships among the taxa.

8. The relationships of species revealed by diploid meiosis is confirmed by similar studies in polyploids.

9. Morphological data is used to provide a classification based on the methods of numerical taxonomy, and a comparison is made with classifications of the group based on other methods.

10. A case is made out for considering all methods of revealing evolutionary relationships in the group equivocal. The relative positions of taxa to one another may reflect the rate of evolutionary change, and whether speciation was allopatric or sympatric, rather than showing evolutionary relationships.

11. A classification is suggested which incorporates the best features of the experimental approach with one formal classification.

ACKNOWLEDGEMENTS

The research described in this thesis was begun in the Botany Department at Durham, under the supervision of Professor D.H. Valentine. I should like to thank him for suggesting this line of research, and for providing material and advice, and for his criticism of the manuscript.

Dr. J.L. Crosby has also given generously of his advice, criticism and time, during the preparation of this thesis, for all of which I am extremely grateful.

Work was continued at Queen Elizabeth Grammar School, Blackburn, and I am most grateful to Dr. F. Tyler and Mr. D.W. Shaw for their understanding and constant encouragement.

The Head of department of the Department of Biological Sciences, Portsmouth College of Technology has also provided facilities which I gratefully acknowledge. I wish to thank Dr. M.A. Carter for his encouragement.

I should like to thank the technical staff of the Department of Botany at Durham for their help at all times. Foremost among these was Mr. J.S. Redhead, without whose co-operation this work would not have been completed.

Mrs. S. Martin, Biological Sciences Department, Portsmouth, photographed and printed Figures 2,3,4, and 5, for which I am very grateful.

Finally I acknowledge receipt of a research studentship from the Department of Scientific and Industrial Research during my time at Durham.

CONTENTS

	Page
Introduction	1
Classification of Primulas (a) Formal	5
(b) Experimental work	8
The genetics of ecological races and species	11
Genetics of Primula	14
Previous investigations	15
The scoring of characters	16
Results. 1. Presence or absence of peduncle	18
2. Length of peduncle	25
3. Flower colour (a) Anthocyanin production	28
(b) Inhibitor of anthocyanin	30
4. Corolla diameter	32
Comparison of corolla diameter in populations of P.veris	33
Corolla diameter in crosses between P.vulg. and P.elatior	35
5. Capsule length	40
Comparison of corolla diameter and capsule length in families H30 and H36	41
6. Hairiness	43
7. Stickiness of seed	47
8. Capsule posture	53

CONTENTS

		Page
Linkage of characters		53
Discussion of Genetics		55
Cytology.	Mitosis	65
	Mitotic chromosomes	67
	Meiotic studies	69
	Chromosomes during meiosis	73
Technique. Interpretation of observations		74
Meiosis in haploid *P.veris*		75
Meiosis in the diploid species		76
Hybrid plants		77
Chiasma frequencies in parental plants and F1 hybrids		
Meiosis in F1 hybrids (a) (*P.vulgaris* x *P.elatior*)		79
Polyploids		91
Meiosis in autotetraploids		93
Autotetraploid *P.elatior* x *P.veris*		99
Comparison of the Primula data with Pearsons model		100
Meiosis in allotetraploid hybrids		102
4n (*P.vulgaris* x *P.elatior*)		103
Meiosis in allotetraploid (*P.vulgaris* x *P.veris*)		105
Meiosis in allotriploid (2n *P.veris* x 4n *P.elatior*)		108
Discussion		117
Conclusions		135

CONTENTS

Page

Appendix A

Presence/absence peduncle scored at different times in H3 F2 (elat. x vul.) i

Presence/absence peduncle scored at different times in H36 F2 (vul. x elat.) ii

H41, H43, H44 and H45-48 iii

Appendix B

Variation in the number of pedunculate inflorescences and basal flowers, in F1 (vulgaris x elatior) and F1 (elatior x vulgaris) iv

Appendix C

Variation of capsule length with corolla diameter F2 (vulgaris x elatior) v

Appendix D

Chromatographic analysis of sugars associated with seed and placenta in P.vulgaris vi

Appendix E

(a) Pairing at meiosis in Primula hybrids vii

(b) Pollen fertility and chromosome pairing in Primula hybrids viii

Appendix F

Data of meiosis in the autotetraploids i. 4n P.elatior ix

ii. 4n P.veris x

CONTENTS

Page

Appendix G

Data of meiosis in (a) 4n (*veris* x *vulgaris*) xiii

(b) 4n (*vulgaris* x *elatior*) xvi

Appendix H

Data of meiosis in the allotriploid (2n *P.veris* x 4n *P.elatior*) xvii

Bibliography xix

INTRODUCTION

"Whenever a biologist wishes to make generalisations about the organisms he studies he is forced to arrange them in groups of some kind", (Sneath, 1962).

A system of classification does not necessarily show anything about the evolutionary relationships of the taxa it describes. Indeed in the days before evolutionary theory was accepted there was no need to assume that any similarity between taxa was due to the type of relationship resulting from having ancestors in common. There need be no theory of relationships before a classification is developed, for naming and classification are aspects of the same process. As Walters (1963) has pointed out, it is impossible to give an organism a name, without indicating that it is different from the organisms to which the name is not applied. Hence, two categories of organism have been established, the organism to which the name is applied and all those to which it is not applied, arising directly out of the linguistic situation, so that classification can be said to go back to earliest times, when the process of naming organisms began.

An important concept in taxonomy is that of the species. Generally the characteristics of a species in formal taxonomy are those listed in Harrison (1963). These are:-

1. Overall resemblance of the constituent individuals.

2. Distinction from other groups of the same kind.

3. Persistence in time.

Harrison quotes Lindley (1832) to show that these concepts are quite old in formal taxonomy. Lindley writes, "A species is an assemblange of individuals agreeing with each other in essential characters of vegetation and fructification capable of reproduction by seed without change, breeding freely together and producing perfect seed from which progeny can be reared. Such are the true limits of species .....".

Clearly taxonomists are concerned with true breeding populations which are prevented in various ways from cross-breeding with other populations or species. The formal taxonomist, in paying attention to morphological differences between groups, is making the assumption, either consciously or unconsciously, that such differences can be taken as indications of barriers to gene exchange. This assumption is not necessarily a valid one, whether many morphological differences are looked for as markers, or only a few. Not only may infertility barriers exist between groups of individuals which are morphologically almost identical, but conversely morphological diversity need not be associated with barriers to gene exchange.

Swain (1963) writes, "Systems of classification do not necessarily embody implications of relationships in their structure, but in fact, all those concerned with plants do employ such concepts to the greatest possible extent compatible with existing knowledge and practical utility".

A classification which is based on one or a few criteria is said to be an "artificial" one, while a "natural" classification is based upon overall resemblance. Linnaeus' system of classification of groups of individuals into species and groups of species into genera, was a natural one for it was based on overall resemblance. The establishment of higher categories was however artificial, for it was based upon the number of parts in the flower.

Modern taxonomy is generally taken to date from Linnaeus, although several of his categories are of much greater age.

Principally, Linnaean classification is based upon morphological features. Of this formal approach Cain and Harrison (1958) write, ".... since .... its results are so much a matter of opinion, some attempt should be made to make zoological comparison in general and taxonomy in particular more precise", Cain (1962) says, "for large parts of the plant and animal kingdom, our best mode of classification is still by 'blind groping'.

Increasingly, work is being directed towards the

production of "natural" classifications, in an effort to uncover evolutionary relationships. Although the 'morpho-geographical' approach to classification is still important in formal taxonomy, more use is being made of other characters of organisms.

Such studies frequently employ the technique of bio-systematics, or experimental taxonomy. For instance, in the group of Primulas studied in this work, Valentine has used crossability data to make deductions about the taxa concerned.

The present work also makes use of the methods of biosystematics. Information about the genetic control of characters which serve to differentiate taxa can sometimes be used to draw conclusions about the status of the taxa concerned; the first part of this work describes attempts to investigate such genetic control.

The characters were mainly those used in the formal classification of the group, but other characters were also considered in an attempt to produce a phenetic classification, using the methods of numerical taxonomy.

Relationships within a group of species can sometimes be deduced from the extent of the pairing of the chromosomes of different specific origins in diploid interspecific hybrids. Data from meiosis in allopolyploids can also be used to evaluate the data from the diploids. These techniques have been employed here.

Classification of Primulas

(a) Formal

The history of the classification of the group of taxa which are the subject of this work, has been a varied one. Several of them have been known since classical times (Smith and Fletcher, 1947), although not necessarily distinguished from one another by different names.

In 1753, Linnaeus following established tradition, regarded what are now Primula vulgaris Huds. the primrose, P. elatior (L) Hill, the oxlip, and P. veris L. the cowslip, as a single species embracing three varieties. During the same century Hudson raised the primrose to specific rank, splitting it off from P. veris, and Hill did the same for the oxlip. Various other taxa were later described, and in 1899 Pax constituted the section Vernales of the genus, to include some of these taxa, but excluding P. megaseaefolia. Further additions to the Vernales were made, and the taxa which have been described as species of the section, and recorded in Smith and Fletcher (1947), are listed in Table 1.

Table 1

Taxa of Primula considered in this work

Taxon	Date	Notes
1. P. veris L	1753	In Species Plantarum.
2. P. vulgaris Huds.	1762	
3. P. elatior (L) Hill	1765	
4. P. amoena Bieb.	1808	Associated with P. elatior by Bieberstein.
5. P. pallasii Lehm.	1817	Specific status accorded by Harrison (1931) after hybridisation experiments.
6. P. megaseaefolia Boiss.	1879	Assigned by Pax to Carolinella; to Megaseaefolia by Balfour, 1913.
7. P. juliae Kusn.	1901	Closely allied to P. meg. - Balfour, 1913.
8. P. intricata Godr. et Gren.	1927	
9. P. lofthousei Harr.	1929	Specific status after hybridisation expts. (1931).

The term taxa has been used deliberately here, for the exact status of the units has been repeatedly argued. Although including all of those listed above in the Vernales, Smith and Fletcher (1947), recognised only six taxa of specific rank, namely P. vulgaris, P. elatior, P. amoena,

P. veris, P. juliae and P. megaseaefolia. Lofthousei, intricata and pallasii are regarded as subspecies of P. elatior. P. amoena and P. megaseaefolia are regarded as doubtful members of the Vernales, but are nevertheless included in it.

A more recent classification of the Primulas is that of Wendelbo (1960). On the basis of pollen morphology he has included all of the above taxa in the single subgenus Primula. He has taken as the type for the subgenus the species which is the type for the genus, namely P. veris. This species is included in the section Primula, which is one of the three sections into which the subgenus is divided, the other two being Megaseaefolia and Juliae.

It can be seen from the foregoing that the treatment of this group of species by the formal taxonomist has been variable, the judgement values of the taxonomists concerned playing an important part in their decisions, which have been based on morphology as a main source of data. Smith and Fletcher (1947) write:- "In contrast with Auricula, where the content and status of the species are practically stable, the widely distributed Vernales offer much more of a field for differences of opinion as to the position of the different units. The occurrence of one or more members of the Vernales in every country in Europe has resulted in

a great mass of literature on the section. Botanists of each country - or even each province or county - for more than three hundred years have contributed their views on the section in general or with particular reference to individual species in their various habitats".

(b) Experimental work

Using experimental techniques, Valentine (1947, 1948, etc.) and his students have endeavoured to produce evidence of the evolutionary relationships of these taxa. Their experiments have involved a great deal of hybridisation between species. The species are heterostyled, and in Valentine's experiments only legitimate crosses were made. "Such crosses, either within or between species are quite compatible so far as fertilisation and initial development are concerned". However, subsequent development of seed is not normal in crosses between species, and the extent and type of abnormalities produced depends upon the direction of the cross. Considering hybrids between P. vulgaris and P. veris:- "The seeds from the cross with P. veris as female are well formed, but their contents vary to some extent from cross to cross. Generally all the seeds are well filled with endosperm, but they are small; their embryos are much smaller than those in non-hybrid seeds, and are sometimes deformed; germination is poor". The symptoms

described in this cross are typical of what Valentine has termed 'type A seeds'.

In the reciprocal cross (P. vulgaris x P. veris), seeds "are large and fairly well formed, but many are empty, and the rest contain only very small amounts of loose, poorly developed endosperm. Dissection of developing seeds shows that in the early stages some endosperm is usually present, and small embryos can be detected in about one-third of the seeds, but these embryos fail to complete their development; only rarely can a small and quite undifferentiated embryo be detected in mature seed. Germination is nil". The characteristics described here are those of what Valentine calls 'type B seeds'. Valentine has used the term "seed incompatibility" for the phenomenon represented by these two types of cross.

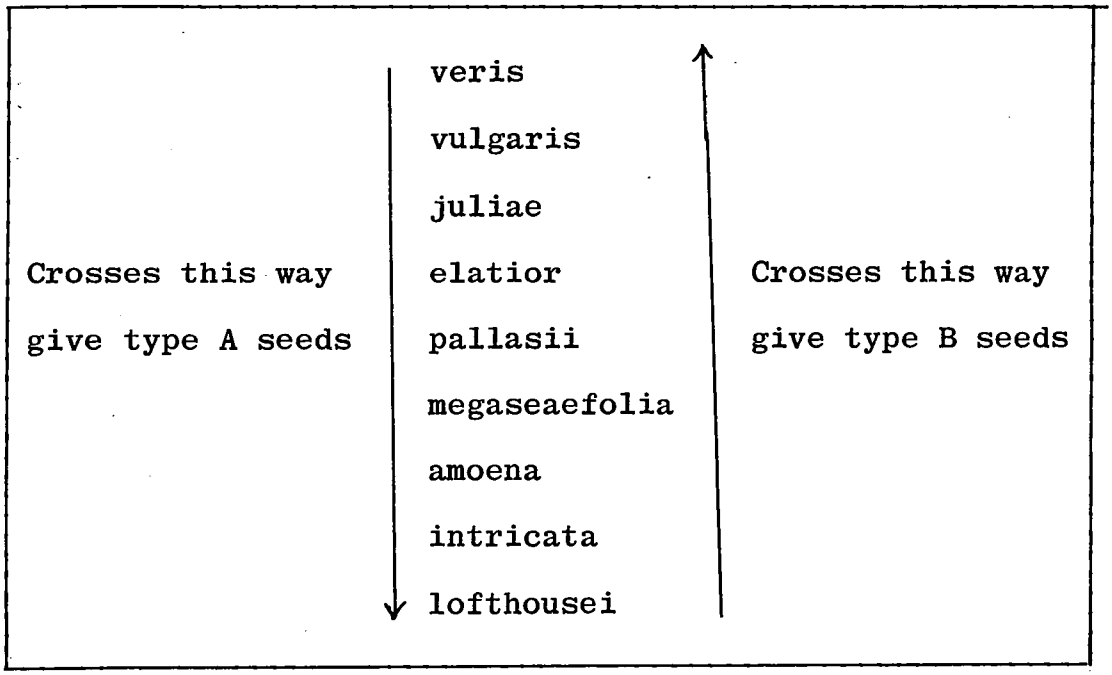
Using the strength of the seed incompatibility reaction between pairs of species, Valentine (1961) has constructed a picture of the relationships of the group, which he claims to be an indication of their evolutionary relationships. This is shown in Table 2.

On the basis of their crossability, Valentine (1951) has placed all the taxa into the category of ecospecies of the same coenospecies, indicating a close degree of relationship.

The experimental,or biosystematic approach has produced a view of the members of this group of taxa as a much more closely knit unit than that of the formal taxonomist. It was therefore considered necessary to conduct further experiments to determine whether or not either of these approaches, the formal taxonomic, or the biosystematic, could be supported.

Table 2

(From Valentine, 1961; Table 3, "The crossability series")



An attempt has accordingly been made in this work to use genetical and cytological observations in hybrids between species of the group at diploid and polyploid level to

approach the question of the relationships of the taxa from yet another point of view.

The genetics of ecological races and species

Clausen and Heisey (1958), after a review of the literature relating to the genetics of specific characters which are used to differentiate taxa in the sweet pea, Avena, Triticum, etc., and an account of their own investigations of the genetics of ecological races of Potentilla glandulosa, were able to draw certain general conclusions. "The available experimental evidence makes it overwhelmingly clear that paired character contrasts between distinct ecological races, subspecies, and closely related species are regulated by systems of genes of moderate complexity". They specify four principal kinds of gene system involved, namely, 1. Additive genes, 2. Epistatic genes, 3. Oppositional genes, and 4. Complementary genes, as well as the unanalysed part of the genotype.

Similar conclusions have been reached by Grant (1956) working with Gilia, and Gajewski (1957, 1959) working with Geum. Grant produced a table concerned with the genetic systems entering into the differentiation of races and species of plants, see Table 3.

The work of these authors, Clausen and Heisey, Grant

and Gajewski, has shown that there is no fundamental difference between the genetic control of factors differentiating races and species. A similar conclusion was

Table 3

Types of Genetic Systems which enter into the differentiation of Races and Species of Plants (from Grant, 1956)

1. Simple Mendelian differences.
(a) Single gene differences.
(b) Complementary factors, etc.
2. Polygenic systems.
(a) Multiple factors with additive effects.
(b) The modifier complex.
(c) Balanced systems with more complex interactions.
3. Cytoplasmic differences.
4. Gene controlled sterility phenomena.
(a) Sterility factors, lethals, etc.
(b) General disharmonies between contrasting genotypes.
5. Chromosomal rearrangements.
(a) Gross structural differences.
(b) Small structural differences.

reached by Mayr (1963) when dealing with animal populations and species ..... . "The results of these hybrid analyses are thus in substantial agreement with those derived from an

analysis of population differences within species".

One line of investigation into the relationships of a group of taxa would therefore be a study of the genetics of factors by which they are recognised as separate entities. The systems of genes controlling the factors can then be compared with the systems found by the other workers, such as Gajewski or Grant. In addition, it may be that some of the genetic mechanisms controlling the characters considered, will be closely alike in some taxa, suggesting that the latter are more closely related to one another than they are to plants which do not have such a mechanism.

Genetics of Primula

The work with new crosses was restricted to hybrids involving P. vulgaris, P. juliae and P. elatior, since F1 hybrids involving these species were available from Valentine's seed incompatibility experiments. Use was also made of hybrids made by Valentine using P. veris.

Crosses were made in an insect-proof greenhouse in the usual way. The plants are heterostyled and only legitimate crosses were made. When the resultant capsules were ripe they were harvested, the products of each plant being kept separately, and scored separately for the characters investigated, (see Table 4). The capsules were stored over winter in dry conditions.

The following spring the seeds were sown in plant-pots in capsule families, the seed from each capsule being kept separate from those of the other capsules. When the seeds had germinated they were transferred to boxes in the greenhouse, and at a later stage were planted outside in the experimental garden. At this time it was found necessary to combine some families where the survival rate did not warrant keeping them apart. Care was taken, however, to ensure that only plants of the same type of cross were put together in these combined families.

Table 4

Characters investigated genetically, and the manner in which the species differ by them

Characters:	Species: vulgaris	elatior	veris	juliae	pallasii	amoena	intricata	lofthousei	megaseaefolia
Peduncle present	-	+	+	-	+	+	+	+	+
" length (cm)	-	30	30	-	37	15	12.5	35	15
Flower colour (yellow/red)	y	y	y	r	y	r	y	y	r
Corolla diam. (mm)	30	18	15	26	20	26	17	17	17.5
Capsule length (mm)	8	14	15	7	(15)	27	15	13	(17)
Pedicel hairy	+	+	+	-	+	+	+	+	+
Seed sticky	+	-	-	-	-	-	-	-	-
Pedicel curved in fruit	+	-	-	-	-	-	-	-	-

Previous investigations

Investigations which are relevant to the present studies

are those of Chittenden (1928), who studied the inheritance of pin/thrum style, degree of hairiness, flower colour, and presence/absence of peduncle; and of Huskins (1929), who took over Chittenden's work when the latter departed for fresh fields. Valentine (1953) added several new fruiting characters, and Clifford (1955) added characters of value to him in identifying hybrids involving P. vulgaris and P. veris in the field. The characters which Clifford examined were again presence/absence of peduncle, and the dimensions of the floral parts. Woodell (1965) has also investigated the genetic control of corolla diameter in populations of P. vulgaris, P. elatior, and their hybrids.

Since Chittenden and Huskins both worked with the same plants, it seemed worthwhile to repeat some of their experiments with different stocks, and if possible to extend their investigations.

The scoring of characters

Each plant was given a code letter of its own, and a diagram of the relative position of each plant in the experiment plot was drawn up, so that the same plant could be scored at different times for different characters, in an attempt to uncover correlated characters.

The characters scored were as follows: at the time of flowering; presence/absence of peduncle; presence/absence

of anthocyanin; corolla diameter. At the time of capsule formation: presence/absence of peduncle; posture of capsule; stickiness of seed; hairiness; capsule dimensions, were recorded. Crossing occurred freely in the open beds, and no difficulty was experienced in obtaining ample ripe capsules and seeds from the majority of plants. The presence/absence of peduncle was scored on each occasion, because it was thought that this could be used as a check on the identity of individual plants. Later it was realised that the phenotypic expression of this character was varying. The manner in which the species differ from one another for the characters studied is shown in Table 4.

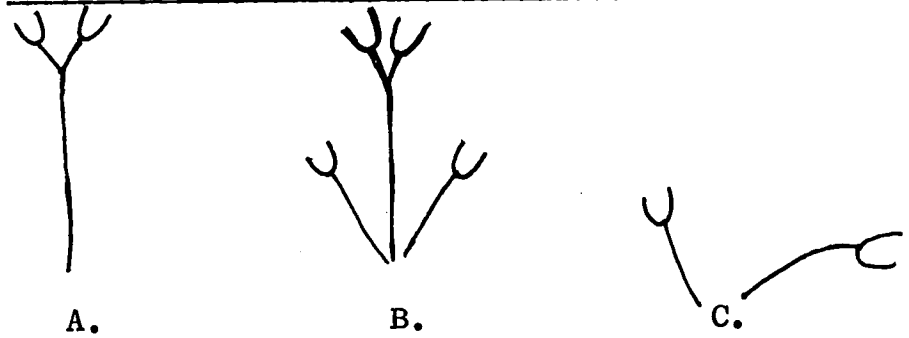
Results

1. Presence or absence of peduncle

P. vulgaris and P. juliae are alike, at least phenotypically, in differing from the majority of species of Primula in not possessing a peduncle. The flowers and capsules are borne in umbels on the top of the peduncle in those plants which possess the character, while the non-pedunculate species usually have no scape. This is one of the characters investigated by both Chittenden (1928) and Huskins (1929), and they came to the conclusion that the character is controlled by a single gene, the pedunculate character being dominant to the non-pedunculate.

However, the condition is not a straightforward one since the F1 bears both pedunculate and non-pedunculate inflorescences on the same plant. Figure 1 shows the types of inflorescence which are referred to in this work.

Figure 1. Types of inflorescence



A. Pedunculate only: found in P. elatior.

B. Both types of inflorescence found in some hybrids.

C. Non-pedunculate only: found in P. vulgaris.

Unfortunately, this is not an easy character to score, although superficially it may appear to be easy. Part of the difficulty is its variation in expression, possibly due to both environmental and genetical influences. This variation is brought out by referring to my data for presence/absence of peduncle scored twice at different times during the same season, in the same plants. Data observed at flowering time and at the time of capsule production are set out in Appendix A. In some cases there are considerable differences, at one time a particular plant showing only pedunculate inflorescences, while at another time it may show either non-pedunculate only, or both types of inflorescence.

In families H30 and 36 F2 (vulgaris x elatior), plants which later had both types of inflorescence at first developed either pedunculate or non-pedunculate only. Of the twenty-three plants observed to do this, nine first of all developed pedunculate inflorescences, while fourteen followed the reverse procedure. This is not significantly different from a one to one ratio, $X^2 = 1.08$, $P = 0.20 - 0.30$, so that there is no evidence for a tendency for one type of inflorescence to develop first in plants which have both types.

Something more of the variation of expression of the character may be seen by referring to Valentine's data

(Appendix B), which records the maximum number of the different types of inflorescences seen in reciprocal F1 crosses over four seasons. The mean number of pedunculate inflorescences per plant is the same in each family, while the mean number of basal flowers in one family is 10.7 and in the other is 13.1. These figures do not differ significantly from one another, since t = 0.004, and the probability of the two being the same is more than 0.90.

What are significant are the extremely high standard deviations for the number of basal flowers, compared with the means. In family G89 where the mean number is 10.7, the standard deviation is 10.19, while G90 and G91 together have a mean number of 13.1, and a standard deviation of 11.7. In both cases there are plants which did not produce any basal flowers at all during the four years. This emphasises the great plasticity of the character.

Some of this variation could be taken into account by scoring the plants, as above, at different times during their development, but since there could be no guarantee of accuracy, it was decided not to separate plants with peduncles into those with, and those without non-pedunculate inflorescences.

The results of scoring families involving crosses

between P. vulgaris and P. elatior are presented in Table 5. Under the heading of "family" is given the code letter of the cross, while under "cross" is represented the constitution of the F1 plants involved in making the F2. For example, (ve x ev) means that an F1 plant with vulgaris as the maternal plant, was used as seed parent, while an F1 plant with elatior as the maternal plant, was used as pollen parent.

Table 5

Peduncle control in F2 families of P. vulgaris x P. elatior

Family	Cross	Ped.	Non-ped.	Seed sown	Plants scored as % of seed sown
H30	(ve x ev)	94	32	160	78.75
H36	(ev x ev)	100	14	131	87.02

On a single gene hypothesis the expected F2 ratio would be 3:1, pedunculate to non-pedunculate. The F2 totals for H30 and H36 are 194 pedunculate: 46 non-pedunculate, and this deviates from expected, (X^2 = 4.34, P = 0.05 to 0.02).

The reciprocal F2 families differ from one another. One, H30 with a ratio of 94 pedunculate: 32 non-pedunculate, shows the three to one ratio expected. The other family

H36, does not show a three to one ratio, (X^2 = 7.73; P = less than 0.01), and deviates markedly from expected. Heterogeneity X^2 = 5.08, P = 0.05 to 0.02, confirming the impression that the F2 families differ significantly from one another.

The backcross data, presented in Table 6, shows a similar type of variation.

Table 6

Peduncle control in (vulgaris x elatior) backcross to P. elatior

Family	Cross	Seed sown	Plants scored as % of seed sown	Ped.	Non-ped.	1:1 ratio hypothesis X^2	1:1 ratio hypothesis Probability
H41	ve x v	54	53.7	6	23	9.96	less than 0.01%
H43	ve x v	88	39.7	9	26	8.24	" "
H44	ve x v	46	52.1	6	18	6.00	" "
H45	ev x v	104	67.3	30	41	1.72	0.1 to 0.2
H49	ve x v	51	25.4	6	13	2.76	0.05 to 0.10

Here again, there is variation between the different families, with two of the families not differing from the

expected 1:1 ratio.

There are differences according to the direction of the cross, for H45 (ev x v) differs significantly from the other families combined (ve x v). Heterogeneity $X^2 = 5.6$, so that the probability that the backcross is homogeneous is less than 0.02.

One possibility which might have explained the differences between the F2 families, and also the backcross differences, would have been the occurrence of cytoplasmic factors. These could have produced reciprocal differences in both F2 and backcross families. For example, the F2 family H36, has as the ovule parents F1 plants formed with P. elatior as the female. Consequently, the background cytoplasm of the female parents of the F2 derives from P. elatior, and in this family there is an excess of pedunculate plants.

In the reciprocal cross (H30), with P. vulgaris providing the background cytoplasm, there is no upset to the expected 3:1 ratio of pedunculate to non-pedunculate plants.

Considering the backcross families however, most of the families with P. vulgaris providing the background cytoplasm, (H41, H43, H44), show deviations from the 1:1 ratio expected. One family, H49, did not show such a

change.

Similarly, the only backcross family with P. elatior providing the background cytoplasm, (H45), does not deviate from expected.

It may be significant that on the occasions when P. vulgaris provides the background cytoplasm, and there is a deviation, then there are more non-pedunculate plants than expected; while in the reciprocal crosses where there is a deviation from expected, there are more pedunculate plants.

However, it does not seem that this can be a straightforward cytoplasmic situation, for some crosses are apparently not affected at all, although made with the same plants.

It is interesting to refer to Chittenden (1928) at this point. His data are for F2 (juliae x elatior). He states, "The difference between pedunculate and non-pedunculate appears to be a single factor difference, although in the F2 particularly there is an excess of pedunculate individuals". In fact, his ratio of 87 pedunculate to 11 non-pedunculate is significantly different from 3:1, (X^2 = 8.85; P = less than 0.01). His backcross to juliae does however give a 1:1 ratio (49 ped.; 46 non-ped.; X^2 = 0.0946; P = 0.7-0.8). Huskins (1929), working with the same stocks says, "In the second generation families, however, there is a rather

large excess of pedunculate plants, the numbers being 87:11, instead of the expected 3:1. The pedunculate condition is one which is greatly affected by the physiological condition of the plant, which accounts for this deviation".

Attempts to produce hybrid families involving P. juliae and P. elatior alone during the present experiments failed, due to the poor germination of the few seeds produced.

My own data for juliae involve a triple hybrid. This was produced by crossing an F1 (vulgaris x elatior) plant with P. juliae. The figures of 50 pedunculate: 36 non-pedunculate do not differ from a 1:1 ratio, (X^2 = 2.26; P = 0.10 to 0.20). This is the ratio one would expect if P. juliae differed from P. elatior by a single recessive factor for the condition. However, in view of the variability of the character already demonstrated, it would be dangerous to assume that the phenotypic resemblance of juliae and vulgaris in lacking a scape is due to similarities at the gene level.

2. Length of Peduncle

Apart from the "straightforward" control of peduncle presence or absence, there appears to be genetic control of the length of this structure. Valentine (1953) says, "it is probable that modifying genes which affect the length of

the peduncle are also segregating as the range of variation in peduncle length is much greater in the F2 families than the parents or the F1 hybrids".

A graph of the variation in the length of this structure is shown in Figure 2 for the F2 families H30 and 36. It can be seen that the variation is both large and continuous. Table 7 shows the coefficient of variation of several families.

Table 7

Variation in peduncle length in hybrids of *P. vulgaris* and *P. elatior*

Family	Number of plants	Mean	Coefficient of variation
F1 (D104 & S24)	7	13.32	19.25
F2 (H30)	83	6.885	59.99
(H36)	90	7.69	37.11
Backcross to *P. vulgaris*	28	4.7	44.67

A 't' test on the means of the two F2 families in Table 7, show that they do not differ significantly from one another for mean peduncle length; $t = 0.0387$, so that the

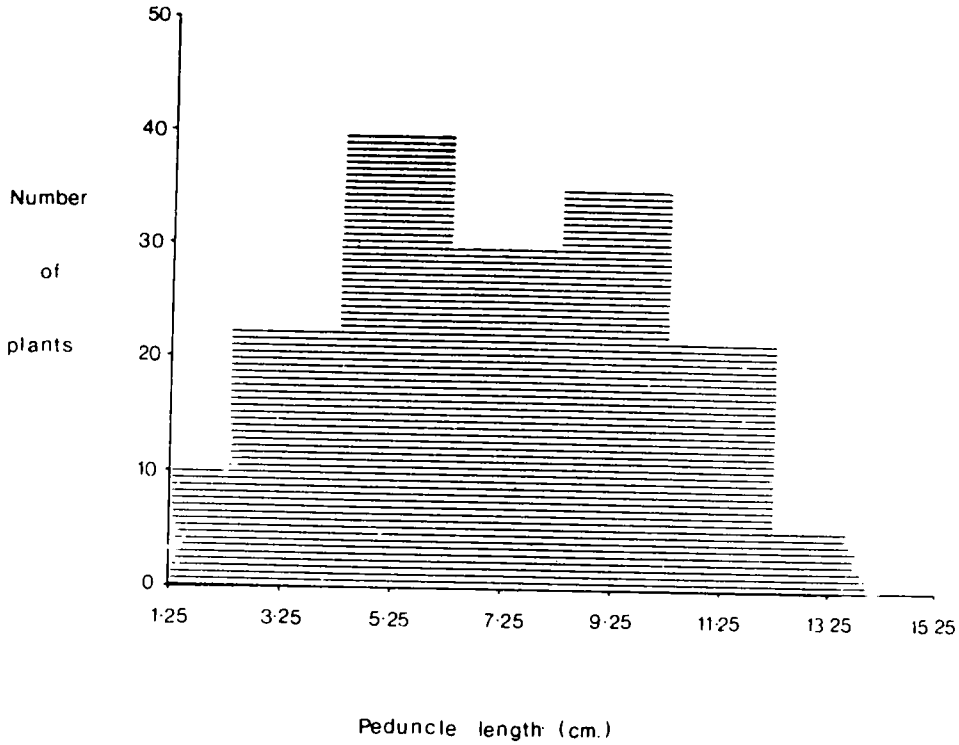


Fig. 2. Variation in peduncle length, F2 (P vulgaris x P elatior)

probability that they are the same is between 0.9 and 0.95.

The table shows that the coefficient of variation is large for both F2 families and the backcross, when compared with the F1 and P. elatior. Taken in conjunction with Figure 2, which shows the continuous nature of variation, this demonstrates the polygenic control of the character.

Another interpretation of the mode of control of peduncle presence/absence is possible. Instead of requiring a single gene for presence/absence, a number of genes could be taken to control the character. In this case there might be a threshold value above which a peduncle would be present, its length determined by the total number of alleles present. Below the threshold value no peduncle would be apparent in normal conditions. Thus, environmental factors such as temperature, might be expected to affect the character if they altered the value of the threshold.

If this hypothesis is correct, then one might expect that occasionally, non-pedunculate parents with allele concentrations just below the threshold, would give rise to pedunculate offspring. The proof of this hypothesis would require further experimental work. Plants of the F2 (vulgaris x elatior) lacking peduncles should be crossed. Occasionally some of them might be expected to give rise to plants with peduncles.

3. Flower colour

(a) Anthocyanin production. Another way in which the species may differ from one another is flower colour. P. juliae has a red corolla, while the other species concerned here usually have yellow, although there are variations. All the representatives of the other species concerned here were yellow.

The yellow pigment is borne in plastids within the cytoplasm, while the anthocyanin responsible for the red colouration is found in solution in the cell sap.

As a result of his crosses, Chittenden (1928) postulated a single dominant gene R for anthocyanin production, and a gene D which intensifies the colour. On this hypothesis one would expect a red F1, a ratio of 3 red:1 yellow in the F2, a ratio of 1 red:1 yellow in the backcross to P. vulgaris, the backcross to P. juliae being all red. The figures obtained from scoring hybrid families between P. vulgaris and P. juliae are recorded in Table 8.

The F1 and F2 families support the hypothesis of a single gene for anthocyanin, the ratio of the latter being exactly the one expected. The backcross to P. vulgaris should give a 1:1 ratio, and the actual ratio of 45:20 differs from expected, (X^2 = 10.4; P = less than 0.01). The backcross to P. juliae can be taken to support the

Table 8

Inheritance of corolla colour in crosses involving juliae and vulgaris

Cross	Red	Yellow
F1 (vulgaris x juliae) A40	A11	-
F2 (" ") H1-8	51	17
(vulgaris x juliae) x vulgaris H10 & H12	45	20
(vulgaris x juliae) x juliae H20	33	1

hypothesis, since it is probably safe to ignore the single yellow plant as an accident, possibly due to a chromosome being lost in a reduction division, or to a stray pollen grain. Crosby (personal communication) has the impression that plants with anthocyanin are more resistant to attack from the fungus Botrytis, and this would possibly account for the higher proportion of plants with anthocyanin than expected in the backcross to P. vulgaris. Only 56.6% of the plants in this family survived to be scored for this character, so that it is possible that this has interfered with the ratios.

The bulk of this data therefore, supports Chittenden's hypothesis of a single gene for anthocyanin production.

No attempt was made to score for the intensifier.

(b) Inhibitor of anthocyanin. Further investigations of Chittenden's involving P. juliae in crosses with P. elatior, revealed the presence of a dominant allele in some plants of the latter species, which inhibited anthocyanin production. Some plants of P. elatior were homozygous for this factor, others were heterozygous, while yet others did not possess the factor at all.

Some yellow F1 plants were available from Valentine's experiments, indicating the presence of the inhibitor in some of his stocks of elatior. The opportunity was therefore taken of making a number of crosses between P. juliae and different plants of P. elatior of separate origins in an attempt to discover something of the distribution of this gene. Unfortunately, although several hundred seed were produced, only a handful of the F1 plants reached maturity, so that this aspect of the work had to be abandoned.

Some experiments with triple hybrids served to demonstrate the presence of the inhibitor in populations of P. elatior, and to confirm its nature. The results of scoring the cross yellow (P. vulgaris x P. elatior) x P. juliae gave 50 red flowered plants to 39 yellow flowered.

Assuming that there were no inhibitors present, one

would expect all the offspring of this cross to be red, since the presence of anthocyanin is dominant to absence. If however, one subscribes to the inhibitor theory then one would expect to obtain a 1:1 ratio red:yellow. The actual results do not differ from expected (X^2 = 1.13; P = 0.20-0.30), so that these results support the idea of a single dominant inhibitor of anthocyanin.

Further confirmation of the presence of the inhibitor in some populations of P. elatior is given by scoring some of Valentine's F1 and backcross families. The results of scoring the hybrids between P. juliae and P. elatior are given in Table 9.

If there were no inhibitors present, all the offspring of these crosses would be expected to have pink flowers, so that the appearance of yellow flowers shows the presence of the inhibitor. Thus, it can be seen from these results that some of the plants of P. elatior do contain the inhibitor. Some of them are homozygous for the factor, so that their offspring produce no red flowers, while others do not contain it at all. It would be interesting to extend the investigation, and find out the effect of the inhibitor on those plants of P. vulgaris which contain anthocyanin.

Table 9

Anthocyanin inhibitor from P. elatior expressed in crosses with P. juliae

Family	Pedigree	Pink Flowers	Yellow Flowers	Note
R115	(EV x J)	21 (all in flower)	Nil	No inhibitor
R119	"	22	0	" "
R127	"	2	0	" " ?
R129	"	3	3	Inhibitor
R134	"	5	2	"
R170	"	11	11	"
P125	F1 (elatior x juliae)	28	0	No inhibitor
P170)) P171)	"	0	37	Homozygous inhibitor

4. Corolla Diameter

Corolla diameter is another character which differs among the species, and which Valentine (1955) considers to be probably under the control of polygenes. There is variation in the diameter of the corolla from the small one of P. veris to the large one of P. vulgaris.

Comparison of corolla diameters in populations of P. veris

The opportunity occurred of comparing corolla diameters in populations of P. veris from different areas, and this was taken to see if the character is as uniform as is generally supposed. Table 10 contains results of scoring plants for this character, and associated statistics, for cowslip populations from Durham, Denmead (Hampshire) and Oxford, the latter data from Woodell (1965).

Table 10

Mean corolla diameters and standard deviations for populations of P. veris

Locality	No. in Sample	Mean (cm.)	S.D.
Oxford (Woodell)	43	1.62	0.1959
Durham	88	1.36	0.1365
Denmead (pins	126	1.02	0.2348
Denmead (thrums	149	1.10	0.1721

Woodell (1965) has shown that there is no difference in his sample between pin and thrum corollas as far as diameter is concerned, and this is so for the Denmead data too. The "t" test of pin/thrum populations from this site gives a result of 1.53, so that the corolla diameters do

not vary significantly between the two types of flower, since the probability that the two means are the same on this result, is between 0.30 and 0.50.

A comparison of populations from different parts of the country again reveals no significance in the small differences between them. Thus, Oxford compared to Durham, t = 0.5359; P that means are from the same population = 0.1-0.2; Durham compared to Denmead thrum, t = 0.4638, P = 0.1-0.2, probability that means are from same population; Oxford/Denmead, t = 0.6316, P = 0.1-0.05, that the means are from the same population.

In all cases the probability is that the means are the same, so that statistically all the populations are the same.

The results of scoring corolla diameter in various hybrid families of P. veris and P. vulgaris are given in Table 11. The P. veris samples came from plants grown in the laboratory grounds at Durham. The samples of P. vulgaris measured were plants taken from wild populations in County Durham by Valentine, and the corollas preserved on cards under transparent tape.

There is continuous variation in the F2 (see Fig. 3), associated with a correspondingly greater coefficient of variation than in the other families. In the F2, 77 pin

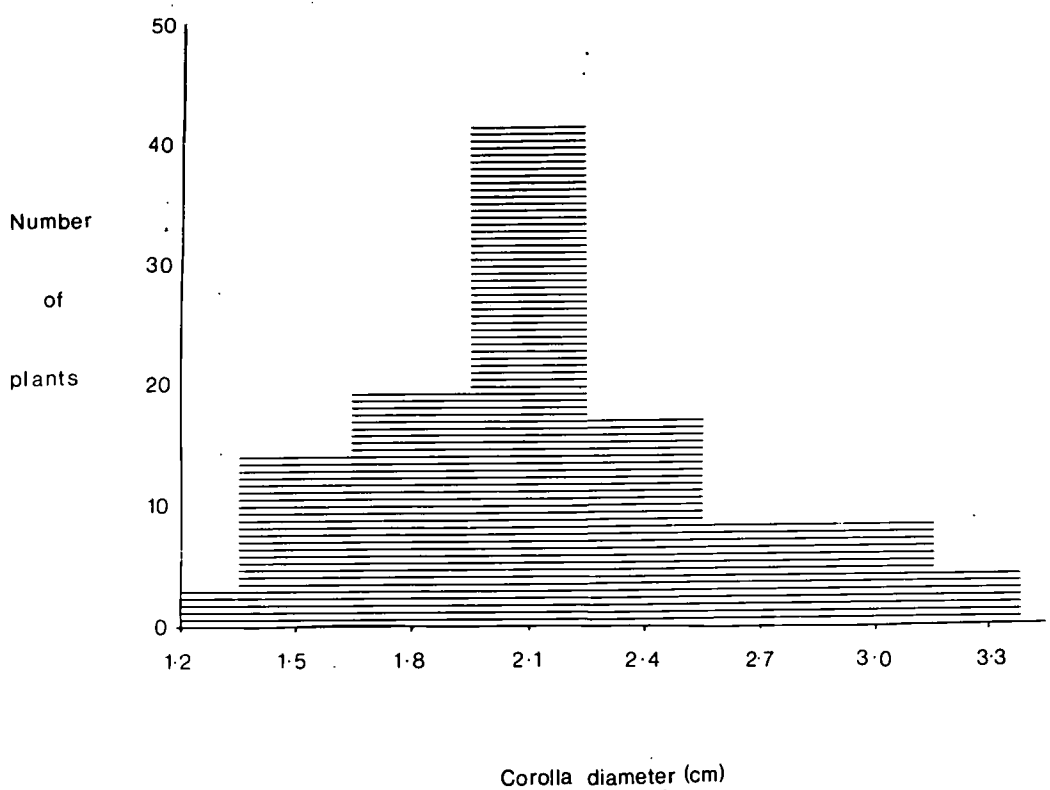


Fig.3 Variation in corolla diameter of F2 (P.veris x P.vulgaris).

flowers were measured against 37 thrum, so that it is necessary to be certain that there is no difference in corolla diameter which might weight the result. However, since "t" = 0.0083, the probability of the two being the same is more than 0.90.

The evidence presented here supports Valentine's contention that corolla diameter is under the control of polygenes in these two species.

Table 11

Corolla diameter in P. vulgaris, P. veris and their hybrids

Family	Number of Plants	Mean Corolla Diameter	Coeff. Variation
P. vulgaris (Valentine's cards)	100	2.8 cm.	9.44
P. veris (Durham garden)	88	1.3	10.03
F1 (veris x vulgaris) G123	55	2.2	1.15
F2 (veris x vulgaris)	77	2.05	21.93 pin
D260 & D173	37	2.2	25.36 thrum
Backcross vulgaris E89	36	2.3	17.29

Corolla diameter in crosses between P. vulgaris and P. elatior

Table 12 shows, in separate sections, measurements of

corolla diameters in F1, F2 and backcross families. The F1 families are of two kinds, depending on whether vulgaris or elatior was used as the female parent. The F2 families had the same F1 male parent (PF3a, Vul. x Elat.), but the female F1 parents differed reciprocally in their own origin. In the backcross, all with P. elatior (F411) as male parent, those with G89 as female are divided according to whether pedunculate or basal flowers were pollinated. This was done in order to determine whether or not the position of the flower had any effect on corolla diameter, due to cytoplasmic factors.

The first point which arises from a study of the results presented in Table 12 is that there are significant differences between the corolla diameters of reciprocal F1 and F2 families. When F1 G89 is compared with the combined F1 families G90 and G91, t = 4.3, and for 38 degrees of freedom, P = less than 0.001. G89, with P. vulgaris as its female parent, has a significantly greater corolla diameter than its reciprocal.

Of the F2 families, H36-38, with P. elatior as the maternal grandmother, has a significantly larger corolla diameter (2.46 cm.), than H32-34 (1.20 cm.), where P. vulgaris was the maternal grandmother, (t = 63.80; D.F. = 38; therefore P = less than 0.001).

Table 12

Corolla diameter in hybrids of *P. vulgaris* and *P. elatior*

(v = vulgaris; e = elatior)

Code	Cross	Mean Corolla Diameter	No. in Sample	Coeff. Variation
F1 G89	(v x e)	2.7 cm.	21	1.611
F1 G90 & G91	(e x v)	2.5	19	8.760
F2 H32	(v x e) x (v x e)	1.2	16	18.745
F2 H36	(e x v) x (v x e)	2.4	25	9.225
Backcross to *P. elatior*:				
L13 & L19	Pedunculate (v x e) x e	2.43	21	32.098
L21 & L23	Basal (v x e) x e	2.40	20	15.796
L15 & L17) L25 & L27)	(e x v) x e	2.46	36	12.408

Moving on to consider the backcross families, two approaches to the problem were investigated, in an attempt to discover if cytoplasmic factors affected the character. All the backcross families were made with an F1 hybrid as the seed parent, and F411 *P. elatior* as the pollen parent.

The F1 plants used as seed parents were either G89 (v x e), or G90 and G91 (e x v). In the case of G89, families were produced from either basal or pedunculate

flowers to find if there were any differences here. Thus, L13 and L19 were produced using only pedunculate flowers of G89 as the seed parent, while L21 and L23 were produced with only basal flowers being used as the seed parents.

A comparison of mean corolla diameters of L13 and L19 with L21 and L23 shows that there is no significance in the slight difference between their means, ($t = 0.00002$; D.F. = 39; P = much more than 0.99). Thus, the position of the female flower, either on a peduncle or not, makes no difference to the corolla diameter of the offspring.

Backcross families produced from crosses using reciprocal F1 seed parents, viz. L15, L17, L25 and L27 - L13, L19, L21 and L23, also showed no difference in corolla diameter; ($t = 0.130$; D.F. = 54; P = more than 0.99).

These results indicate that the differences in corolla diameter are not reciprocal and are not therefore due to cytoplasmic, or maternal factors of some sort, contributed by one of the species taking part in the crosses. This confirms what was hinted at in the F1 and F2 families, where first of all the larger corolla diameter is produced with P. vulgaris as the female parent, and then P. elatior providing the background cytoplasm.

For an explanation it is necessary to refer to the coefficient of variation of the F2 families, and to compare

them with the coefficient of variation for the other families. Both F2 families have very low coefficients of variation considerably lower than those found in the backcross in some cases, and comparable with those found in the F1. This is not what is expected with a large number of genes segregating, but the variation in the backcross demonstrates that large numbers of genes are effecting corolla diameter. The fact that only a comparatively small variation exists in corolla diameter, although this is under the control of a large number of genes, may indicate that there is some correlation between the genes for corolla diameter, and whatever is responsible for the deaths in the F2 families.

Alternatively, it was pointed out earlier, when discussing the scape, that the respective F2 families are variable in the time of production of peduncles, and this is true also of flower production. Consequently, only sixteen plants in family H30, and twenty-four plants in family H36, were scored for corolla diameter, and this may not be a representative sample, for the numbers of plants scored for presence/absence of peduncle were 118 and 119 respectively.

It is therefore very unlikely that the samples actually scored were representative of the population as

a whole, and it would be interesting to investigate the matter more thoroughly.

5. Capsule length

Among other fruit characters, Valentine (1953) investigated capsule length, and this was another character investigated again in these crosses. Details of this character for P. vulgaris, P. elatior and their hybrids, are shown in Table 13.

Table 13

Capsule length in species and hybrids of P. vulgaris and P. elatior

	No. in Sample	Mean Capsule Length	Coeff. Variation
P. vulgaris (G101)	8	0.81 mm	11.44
P. elatior (D7, D13)	10	1.42	15.68
F1 (vulgaris x elatior (D104 & S24)	37	1.1	8.7
F2 (vulgaris x elatior (H30)	144	1.17	18.74
F2 (elatior x vulgaris) (H36)	135	1.07	19.86
Backcross to vulgaris (H44, 45-48, 49)	125	1.14	17.78

Here again the F2 families combined have a much greater coefficient of variation than the F1 and parental generations, and this is continuous, (Fig. 4). This demonstrates the polygenic control of the character noted by Valentine.

However, these experiments reveal differences between the two F2 families, and these are significant, t = 63.9; D.F. = 39; P = less than 0.001 of the two being the same.

Comparison of corolla diameter and capsule length in families H30 and H36

Since both length of capsule and corolla diameter appeared to show similar deviations in reciprocal F2 crosses, the relationship of these two characters was investigated further.

In family H30 the matter is straightforward, for it is clear that the corolla diameter and capsule length do vary together, the one increasing by unit amount whenever the other shows a unit increase. The data are set out in Appendix C, and it can be seen that the other family, H36, is much more variable, and does not show a regression. A 't' test on the significance of the regression in this family gives t = 1.1399; D.F. = (n - 2) = 25; therefore, P = 0.30 — 0.20

In order to show a significant regression, t must be 2.06 or more. Hence H36 does not show a regression of

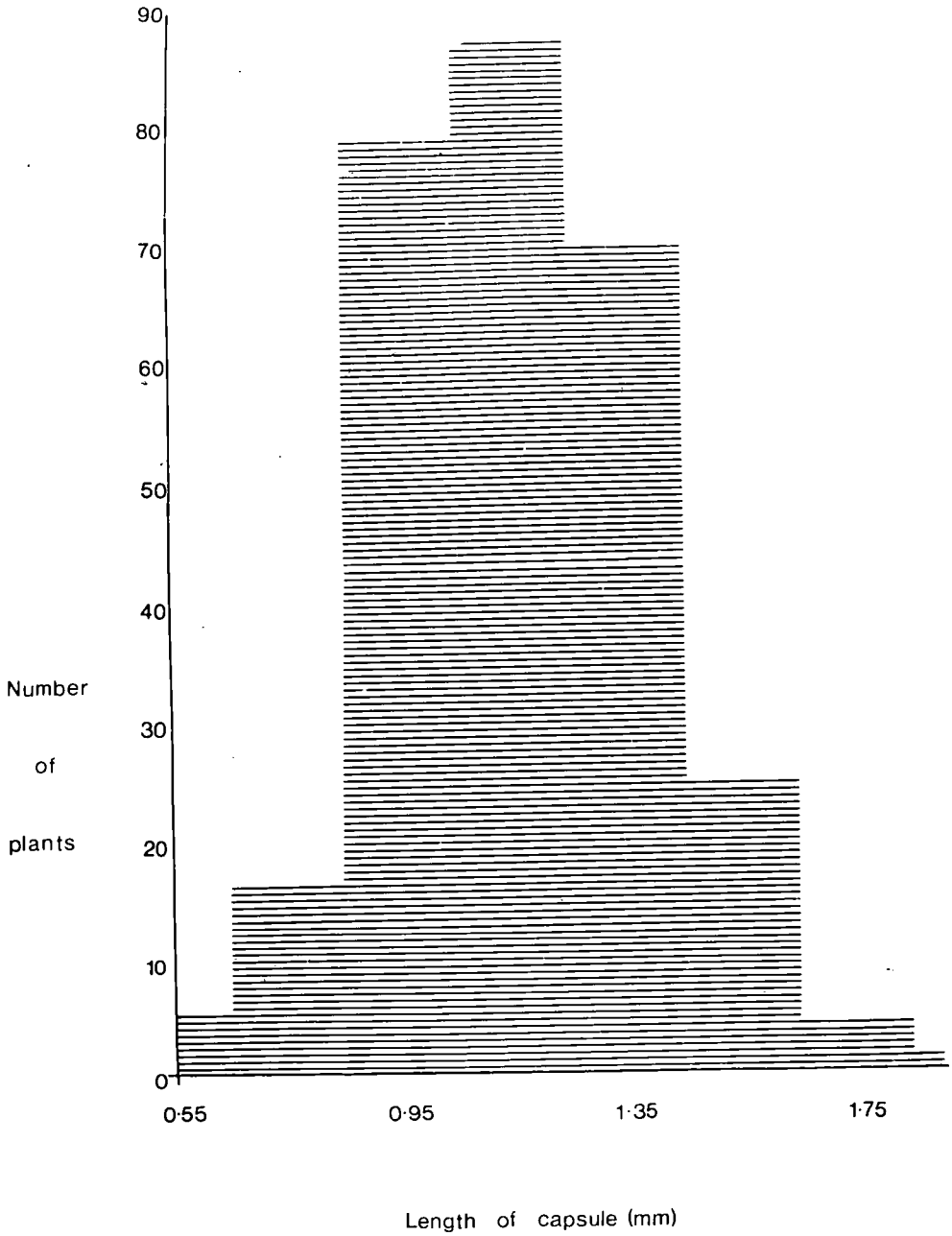


Fig 4. Variation in capsule length, F2 (vulgaris x elatior)

corolla on capsule length, and so differs from H30.

Such a difference between reciprocal F2 families is not expected from the normal segregation of nuclear genes. It is also difficult to accept the mechanism of cytoplasmic control of the characters, however, for it has already been demonstrated that there is polygenic control of both, which by its nature is unlikely to be cytoplasmic.

It is also unlikely that there is a correlation between the deaths due to seed incompatibility, and the control of corolla and capsule sizes. This is because there is no theoretical reason why there should be differences in the percentages of deaths in reciprocal F2 families, due to this reason.

Nevertheless, there have been great differences in survival rates between these two families. Of the plants compared for corolla diameter and capsule length the 64 plants of H30 represent 40% of the seed sown, while the 27 plants of H36 represent 20.6% of the seed sown.

According to Grant (1967), "Genes determining morphological characters which are first expressed in the late stages of development of higher plants, particularly in the fruit and flowers, are frequently linked with genes affecting growth and vigour in early development stages. Related plant species often differ allelically with linked

morphological and viability genes, as indicated by the evidence of artificial hybridisations in many groups".

Grant's so-called M-V linkage system is found in related species of Mimulus, Gossypium, Lycopersicon, Triticum, Tragopogon, and Phaseolus, on homologous chromosome segments. It is often found associated with "rearranged segments differentiating related species of Clarkia, Gossypium, Zea, Triticum and Gilia". It "appears to be a general feature in the architecture of plant species".

If this system exists in Primula it is probably different from the seed incompatibility system of Valentine, and the present data may hint at its presence.

While it would be premature to advance the Primula case as another example of an M-V linkage system, it is tempting to draw attention to it in this context. If there is such a system present, then it is in addition to the seed incompatibility mechanism, and would presumably serve as a means of bringing about stabilising selection. In other words, the inviability of certain genotypes in the F2 would be due to their unfavourable gene combinations. The survivors, on the other hand, possess favourable gene combinations.

6. Hairiness

A character investigated by both Chittenden (1928) and

Huskins (1929) is that of hairiness of the pedicel. P. vulgaris and P. elatior, which they used in crosses with P. juliae, are both very hairy, while P. juliae is glabrous or almost so. Chittenden scored P. vulgaris and P. juliae and their hybrids with the results presented in Table 14.

Table 14

(From Table 2, Chittenden, 1928)

Hairiness in P. vulgaris, P. juliae & their hybrids

Family	Short hairs or none	Slightly hairy	Very hairy
P. acaulis (= vulgaris	-	-	all
P. juliae	all	-	-
F1	all	-	-
F2	29	34	8
Backcross to P. vulgaris	14	47	126
Backcross to P. juliae	28	-	-

Chittenden writes, "In the acaulis x juliae figures, one can get a fairly good fit with the observed numbers by assuming the presence of three factors; two factors N and L for hairiness contributed by acaulis and a factor X contributed by juliae which inhibits hairiness, but only

partially if either N or L is homozygous".

Chittenden gives no indication, other than his column headings, of the standards he used to score his plants, so in our experiments the standards illustrated in Plate 1 were arrived at after examining a number of plants, and were taken as arbitrary standards against which the plants could be scored.

The results of scoring P. vulgaris, P. juliae and their hybrids are presented in Table 15.

My analysis of the species and the F1 agrees with Chittenden. The F2 plants scored number only thirteen, and adopting Chittenden's hypothesis, the expected numbers would be 9.97 short hairs or none:2.48 slightly hairy: 0.203 very hairy. The actual numbers of 11:2:0, do not differ significantly from expected, $X^2 = 0.37$; $P = 0.05$-0.10. Chittenden's data for the F2 gives $X^2 = 4.6$; $P = 0.02$-0.05.

The backcross to P. juliae might be expected to be composed of plants with short hairs or none, and the appearance of plants in the other categories is not expected. However, Chittenden also experienced this type of result, and as the character is one which might be easily influenced by environmental conditions or chromosomal additions, these plants will be ignored.

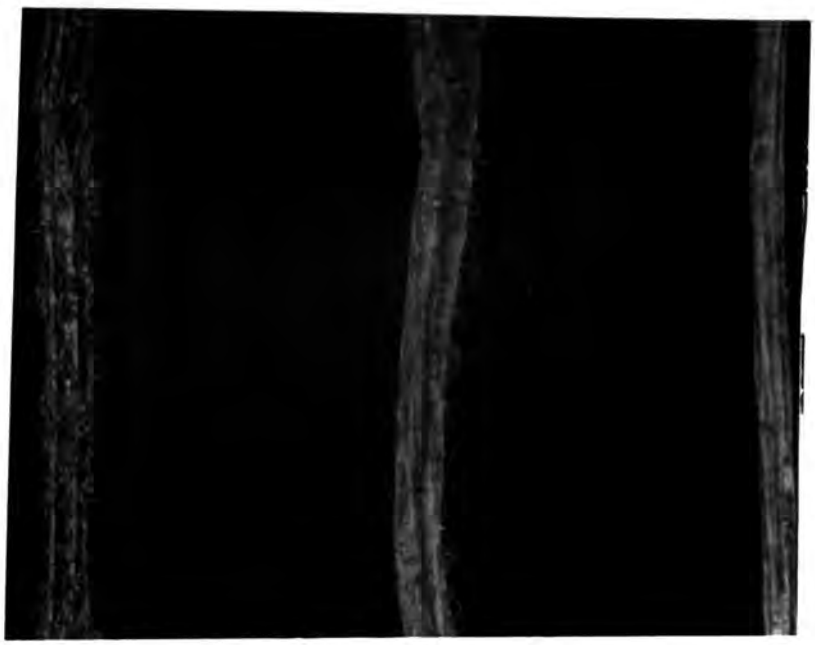


Plate 1. Standards of hairiness used when scoring plants for this character.

A: Very hairy.

B: Slightly hairy.

C: Short hairs or none.

Table 15

Hairiness in crosses involving *P. vulgaris* and *P. juliae*

Family	Short hairs or none	Slightly hairy	Very hairy
P. vulgaris	-	-	all
P. juliae	all	-	-
F1	all	-	-
F2	11	2	-
Backcross to *vulgaris*	14	25	11
Backcross to *juliae*	28	2	2

The backcross to *P. vulgaris* might be expected to give the following results:

$$\frac{NLX}{NLx}\ \frac{NLx}{NLx}\ \frac{NlX}{NLx}\ \frac{Nlx}{NLx}\ \frac{nLX}{NLx}\ \frac{nLx}{NLx}\ \frac{nlX}{NLx}\ \frac{nlx}{NLx}$$

very hairy : slightly hairy : glabrous

2 : 4 : 2

In other words, a 1:2:1 ratio is expected. The actual result of 14:25:11 comes up to expectations, $X^2 = 0.36$ and P = more than 0.8 for 2 degrees of freedom. Chittenden's backcross data differ from expected in this case, but my data demonstrate that his hypothesis to explain the inheritance of hairiness is probably a valid one.

7. Stickiness of seed

It may be seen from Table 4 that one of the specific characters of P. vulgaris is its possession of sticky seeds and placenta. This character is taken to be of value to the plant in assisting in the dispersal of the seeds, for ants collect them from the plant no doubt attracted by the sticky secretion.

Valentine has suggested (1953), that this secretion is sugar, and this was confirmed in the present study by chromatographic analysis (see Appendix D) when the sugars glucose, fructose and sucrose were identified. The validity of this observation is further increased by Percival's (1961) record of just these sugars in the nectar of P. vulgaris.

After examining hybrids of P. vulgaris with P. elatior, Valentine (1953) states, "that several genes are concerned in the formation of sticky seeds and placenta is indicated by the fact that of thirty-nine F2 plants scored, only one showed this character".

In order to test the genetic control of this character further, hybrids between P. vulgaris and P. elatior, and P. vulgaris and P. juliae were scored. The results of scoring P. vulgaris, P. elatior and their hybrids are presented in Table 16.

Table 16

Stickiness of seed and placenta in species and hybrids of P. elatior and P. vulgaris

	Seed sticky	Seed not sticky
P. vulgaris	all	-
P. elatior	-	all
F1 (vulgaris x elatior)	-	all
F2 H30 (v x e) x PF3a	19	92
H36 (e x v) x PF3a	18	87
Backcross:		
H41, 44 and 49; (v x e) x v	40	21
H45 (e x v) x v	55	7

The hypothesis of a single recessive gene would require a dry F1, which has been found, a 3:1 ratio of dry to sticky in the F2, and a 1:1 ratio in the backcross to vulgaris.

The F2 group H36 is in agreement (X^2 = 3.66; P = 0.05-0.10), but H30 deviates from expected (X^2 = 5.00; P = less than 0.05), and the combined families H30 and 36 also deviate from expected (X^2 = 7.13; P = less than 0.01). The backcross families do not give the 1:1 ratio expected on the hypothesis, H41, H44 and H49 (X^2 = 5.90; P = 0.02-0.01),

H45 (X^2 = 36.27, P = less than 0.001). The single gene hypothesis is not supported by this data.

The best fit with the F2 results is obtained by assuming three recessive genes for stickiness contributed by P. vulgaris. When all the genes are heterozygous, as in the F1, then the plant does not have sticky seeds. If, however, one of the genes is present in the homozygous recessive state, and recessive alleles of the other two genes are present, then the plant has sticky seed and placenta. Thus, the F1 combination $\frac{abc}{abc}$ is not sticky, but the combinations $\frac{abc}{Abc}$; $\frac{abc}{ABc}$; etc., are sticky.

On this hypothesis the F2 would be expected to produce a ratio of 54:10, sticky:not sticky, and the actual ratio of 179:37 does not differ from expected, (X^2 = 0.20; P = 0.5-0.7).

The backcross to P. vulgaris should give a 7:1 ratio, and the actual ratio (99:28) differs significantly from expected; (X^2 = 5.77; P = 0.02-0.01).

It was mentioned earlier, in connection with the scoring of peduncle presence/absence, that the high death rate could have played havoc with the results, and the same possibility holds good with this character. Considering pedunculate plants alone, then there is a total of 177 of them in the two F2 families. One would expect on the

single gene hypothesis to obtain 132.75:44.25 not-sticky: sticky, but the actual results differ from this; 145:32; $X^2 = 4.25$; $P = 0.05\text{-}0.02$.

Assuming that all the seeds had germinated and grown to maturity, then there would have been expected on the hypothesis 218.25:72.75 sticky to non-sticky. It is likely therefore, that there have been large losses in both categories, but there is nothing to indicate that these losses have not been unequal. It appears then, that the results of analysing this series of crosses are inconclusive. They do not satisfactorily settle the matter either way, neither supporting Valentine's hypothesis nor contradicting it. Perhaps the most sensible course is to recognise this fact, and to undertake further experiments to settle the matter finally.

The data for crosses between P. vulgaris and P. juliae do not throw any clearer light on the problem, in fact, they confuse the matter still further. The results of scoring these crosses are set out in Table 17.

Table 17

Stickiness of seed and placenta in crosses between *P. vulgaris* and *P. juliae*

	Seed sticky	Seed not sticky
P. vulgaris	all	-
P. juliae	-	all
F1 (A40)	-	all
F2 (H1-8)	11	-
Backcross to *P. vulgaris* (H10 & H12)	63	1
Backcross to *P. juliae* (H20)	16	15

These results are obviously different from those obtained from the crosses involving *P. elatior* and *P. vulgaris*, and do not suggest several genes controlling the character. Rather do they suggest a single gene, at least partly dominant. A single dominant gene requires a sticky F1, which does not occur. The F2 would be expected to give a ratio of 3 sticky:1 not-sticky, and 11:0 does not differ from this by a significant margin, (X^2 = 0.2; P = 0.50-0.70). The backcross to *P. juliae* is, as expected, a good 1:1 ratio (X^2 = 0.2; P = 0.50 to

0.70). The backcross to P. vulgaris can be taken to be in agreement with the hypothesis, since the single non-sticky plant could be due to a mis-score. Only the F1 differs from expected.

It does seem, therefore, that these two species differ from one another by only a single gene of incomplete penetrance, rather than the several genes which separate P. vulgaris and P. elatior. F1 plants may differ from one another for this character, even in non-reciprocal crosses, for the F1-type plants in the F2 must be sticky to give the recorded results, even though the F1 itself is not sticky. It may be therefore, that the gene or genes for stickiness are themselves acted on by modifying genes. This is a question which will require further investigation before firm conclusions about it can be reached.

What are possibly similar types of situation have been described by Clausen (1958). He found that in ecotypes of Layia .... "Shifts of dominance occur, and ratios of segregation vary from hybrid to hybrid .....". For example, presence of central stem is recessive in the cross Jenner x San Bernardino, but is dominant in the cross Cambria x Pala. "Dominance is also reversed in the inheritance of orientation of branching. Ratios such as 43:21 and 9:7 indicate the presence of complementary genes,

whereas 13:3 suggests the interaction of genes having opposite effects. The genotypes are therefore not directly related to the phenotypes".

8. Capsule posture

This is another characteristic of P. vulgaris which was investigated with somewhat variable results. The posture of the fruiting capsule in P. vulgaris is distinctive. In fruit, the capsule is bent down to the ground, due to the curvature of the pedicel. The majority of the other species have their capsules erect. Doubtless, it is of great value to P. vulgaris that its capsule should assume this position, since it enables ants to collect, and so disperse, its sticky seeds.

Attempts were made to investigate the posture of the capsule in various hybrids involving P. vulgaris, but apart from the observation that the character was in fact segregating, little progress could be made. Later crosses made by Valentine revealed differences in capsule posture between reciprocal F1 crosses, suggesting that at least some of the expression of this character is affected by cytoplasmic factors.

Linkage of characters

Although investigations into the relationships of

different genetic characters were made, no correlations, other than those already discussed, were detected.

Discussion of Genetics

There are on record a number of reports of investigation into the genetics of characters by which species, or lesser categories, may be distinguished one from another.

In formal taxonomic terms species are recognised as being separate from one another on the basis of several characters. For instance, Clausen (1951) uses seventeen characters to analyse the differences between species of Layia. Investigations in both Europe and America have thrown light on the genetic control of this type of character. For example, Prazmo (1965) has investigated the inheritance of traits distinguishing different complexes of Aquilegia. Traits "such as; height of plant, shape of leaves, length of spurs, degree of their curvature, dimensions of petals and sepals, length of the androecium, length of the follicles, number of follicles, number of ovules, size of the seeds, time of flowering", were shown to exhibit a continuous variation, "presumably caused by the segregation of polygenes". "On the other hand, such additive and diagnostic characters as the presence or absence of spurs, straight or curved spurs, positioning of the flowers, either nodding or erect, and the flower pigmentation are dependent on one or only a few pairs of allelomorphs".

Doroszewska (1965) found that characters differentiating Trollius chinensis and T. europaeus, such as colour and shape of flowers, dimensions of perianth segments, follicles and beaks, "are all controlled by cumulative multiple factors, and demonstrate in the F2 a continuous variation".

The same types of genetic control have been shown to be operating in this group of Primulas, and indeed one can go further. One can associate the types of genetic systems operating in Primula with those noted by Grant (1956), and shown in Table 3.

Table 18 shows the types of genetic systems which enter into the differentiation of the Primula species, and this can be compared directly with Grant's table, (see page). It can be seen at once that Primula fits neatly into the generalised picture, being differentiated in most of the ways mentioned by Grant. Section 5 of his table will be dealt with after the cytological results have been presented.

In general, these results agree with the observations of the various workers quoted above. The types of genetic mechanism illustrated here are those which separate "distinct ecological races, subspecies, and closely related species", so that Valentine's view of this group being ecospecies of the same coenospecies is strongly supported.

Features such as those investigated genetically are

Table 18

Types of genetic systems entering into the differentiation of Primula species

1. Simple Mendelian differences.
   (a) Single gene differences: e.g. anthocyanin production.
   (b) Complementary factors: e.g. hairiness.
2. Polygenic systems.
   (a) Multiple factors with additive effects: e.g. peduncle length, corolla diameter, capsule length.
   (b) Modifiers .... dominance effected by modifiers in stickiness of seed.
3. Cytoplasmic differences: e.g. capsule posture.
4. Gene controlled sterility phenomena.
   Sterility factors: e.g. Valentine's seed incompatibility.

just the kind of morphological features which are used by the formal taxonomist in separating his taxa, and relating them to one another. For instance, Wendelbo (1960) comments particularly on the uniformity of pollen morphology in his subgenus Primula. Something of the variations of classification which are possible when using the same morphological data were described earlier in this work with

reference to the group of plants being studied, and it is possible to align the taxa in different ways according to the characters chosen for emphasis as being important diagnostically.

The great drawback to this weighting of characters is that it introduces a subjective element. However, in those cases where a phylogenetic check is possible, via the fossil record, there is justification for it. Sporne (1956), for example,has calculated an "advancement index" for all families of Dicotyledons, attempting to use the fossil record and character correlations in order to distinguish between primitive and advanced characters.

Between closely related species it is often difficult to decide between what should be 'advanced' and 'primitive', for the type of character considered by Sporne will be held in common by all taxa. It is the difficulty of deciding which characters should be emphasised which have led to the difficulties of Primula classification.

According to Sokal and Sneath (1962), "In recent years a number of authors have pointed out the logical fallacies underlying attempts to force phylogenetic criteria on to taxonomic groupings, or to arrive at taxonomies based on phylogenetic deductions". They urge a return to the Adansonian postulates of all characters having equal weight,

the taxa being based on correlations between features. "These concepts are the basis for the numerical analysis of taxonomic resemblances. Acceptance of equal weighting allows the ready mathematical treatment of characters, and the use of estimates of resemblance (rather than key characters) for creation of taxa allows for the formation of 'natural' taxonomic groups".

Sneath (1962) further describes the process of numerical taxonomy. "Adansonian classification may be briefly summarised as follows:-

(1) The ideal 'natural' taxonomy is that in which the taxa have the greatest content of information, and which are based on as many features as possible.

(2) Every feature is of equal weight in constructing 'natural' taxa.

(3) Overall similarity (affinity) is a function of the proportion of features in common.

(4) Distinct taxa are based on correlated features.

(5) Affinity is treated as independent of phylogeny, i.e. as an independent taxonomic dimension, and is therefore phenetic".

Ideally, as large a number of contrasting characters as possible should be scored for presence or absence. The characters scored in the present study are listed in Table

Table 19

Nineteen characters of Primula species tabulated and compared

	pallasii	megaseaefolia	intricata	amoena	juliae	vulgaris	elatior	lofthousei	veris
With stolons	-	-	-	-	+	-	-	-	-
Lvs. persist over winter	-	-	-	-	+	+	-	-	-
Lvs. orbicular	-	+	-	-	+	-	-	-	-
Inflorescence pedunc.	+	+	+	+	-	-	+	+	+
Inflorescence spreading radically	-	-	-	-	+	+	-	-	-
Pedicels glabrous	-	-	-	-	+	-	-	-	-
Calyx campanulate	-	-	-	-	-	-	-	-	+
Corolla normally yellow	+	-	+	-	-	+	+	+	+
Corolla limb markedly concave	-	-	-	-	-	-	-	-	+
Corolla orange at throat	-	-	-	-	-	-	-	-	+
Capsule much elongated	+	-	-	+	-	-	+	+	+
Placenta and seeds sticky	-	-	-	-	-	+	-	-	-
Petiole ± well marked	-	+	-	-	+	-	-	-	-
Lvs. broad 1 cm. behind tip	+	+	+	+	+	+	+	-	-
Corolla diam. less than 20 mm	+	-	-	-	-	-	+	+	+
Calyx teeth relatively short	+	-	+	+	+	-	+	+	+
Petals relatively narrow	-	+	+	+	+	-	+	+	+
Lvs. shiny	-	+	-	-	+	-	-	-	-
Ripe capsule erect	+	-	+	+	-	-	+	+	+

19, together with the ways they vary in the different species.

The species may now be compared in pairs to decide how many characters each pair has in common. In this analysis only positive correlations - that is, two pluses - are counted. It is held that two negatives, showing that the two species under consideration both differ from the character stated, does not necessarily indicate similarity. The absence of a specified state does not automatically place them in the same category, for the negative condition may take several forms. To take a hypothetical case, if the character being considered were 'flowers red', then the absence of this colour could be due to the flowers being white or yellow, and to score the negatives as indicating the same state would be incorrect.

Table 20 shows the number of positive characters held in common by any pair of species.

From these data it is possible to construct a dendrogram to express the relationships of the taxa. Sokal and Sneath point out that this is not a family tree, but merely indicates the relationships of adjacent taxa.

The methods used to adjust the taxa to their positions in the dendrogram are collectively known as cluster analysis. For example, the species are retabulated and listed according

Table 20

Numbers of the nineteen characters held in common by any pair of species

	pallasii	megaseaefolia	intricata	amoena	juliae	vulgaris	elatior	lofthousei	veris
pallasii	-	2	5	5	2	2	7	6	6
megaseaefolia		-	3	3	4	1	3	2	2
intricata			-	5	3	2	6	5	5
amoena				-	3	1	6	5	5
juliae					-	3	3	2	2
vulgaris						-	2	1	1
elatior							-	7	7
lofthousei								-	7
veris									-

to their similarities, Table 21. To begin with the species other than elatior were considered, because its inclusion in the early clustering is confusing.

Considering the relationships of P. veris, then it has seven points in common with its closest relative, P. lofthousei. Veris has six points of resemblance with

Table 21

Species pairs in order of similarity

Similarity	Pairs
(7)	elat. - loft.; elat. - pall.; elat. - veris; veris - loft.;
(6)	pall. - loft.; elat. - intri.; elat. - amoena; pall. - veris;
(5)	pall. - intri.; pall. - amoena; intri. - loft.; intri. - veris; amoena - loft.; amoena - veris;
(4)	meg. - juliae;
(3)	meg. - intri.; meg. - amoena; meg. - elat.; intri. - juliae; amoena - juliae; juliae - vulg.; juliae - elat.;
(2)	pall. - juliae; pall. - meg.; pall. - vulg.; meg. - loft.; meg. - veris; intri. - vulg.; juliae - loft.; juliae - veris; vulg. - elat.;
(1)	meg. - vulg.; amoena - vulg.; vulg. - veris;

pallasii, and five with intricata and amoena. P. lofthousei, with five points in common with intricata and amoena has six in common with pallasii. Hence, the order of relationship

is veris - lofthousei - pallasii - and either amoena or intricata. Veris has as its most distant associate P. vulgaris, with only one point of resemblance in common.

Starting from vulgaris, then the picture outlined above is confirmed, in that veris and lofthousei are among the most distant of its relatives with only one point of resemblance each. P. juliae is the closest relative of vulgaris, but not so very close morphologically speaking, with only three points of resemblance. Juliae's closest associate is megaseaefolia, the two species sharing four points of resemblance. Megaseaefolia has three points in common with both intricata and amoena, making a decision about the relative positions of the two species impossible. Indeed the only evidence is offered by the number points in common between these two species and P. vulgaris. The latter species has two points in common with intricata, but only one with amoena. Nevertheless, it is felt that this evidence is too slight to position the species with confidence, so that their exact positions are still debatable.

Primula elatior has seven points in common with each of veris, lofthousei and pallasii, and six with amoena and intricata. With the other species it has fewer points of resemblance, so that it clearly belongs to the veris-lofthousei-pallasii group. Elatior has more points in common with amoena (6) and intricata

(6), than has veris (5), or lofthousei (5), or pallasii (5). Similarly, elatior has more points in common with megaseaefolia (3), and juliae (3), than has any of the species veris (2 & 2), lofthousei (2 & 2), and pallasii (2 & 2). This suggests a species order of veris, lofthousei, pallasii, elatior, amoena, intricata. However, veris and elatior have in common seven points, while pallasii and veris have only six. Consequently, in view of this discrepancy, the position of elatior must be regarded as tentative.

A dendrogram of relationships is shown in Fig. 5. Although it would be dangerous to accept the finer details of relationships indicated here without further support, it is possible to observe the extremes. Vulgaris and veris are phenotypically the most distant pair of species, with juliae closer to vulgaris and lofthousei closer to veris. Consequently, this evidence tends to favour the view associated with the cytological evidence, rather than that from seed incompatibility.

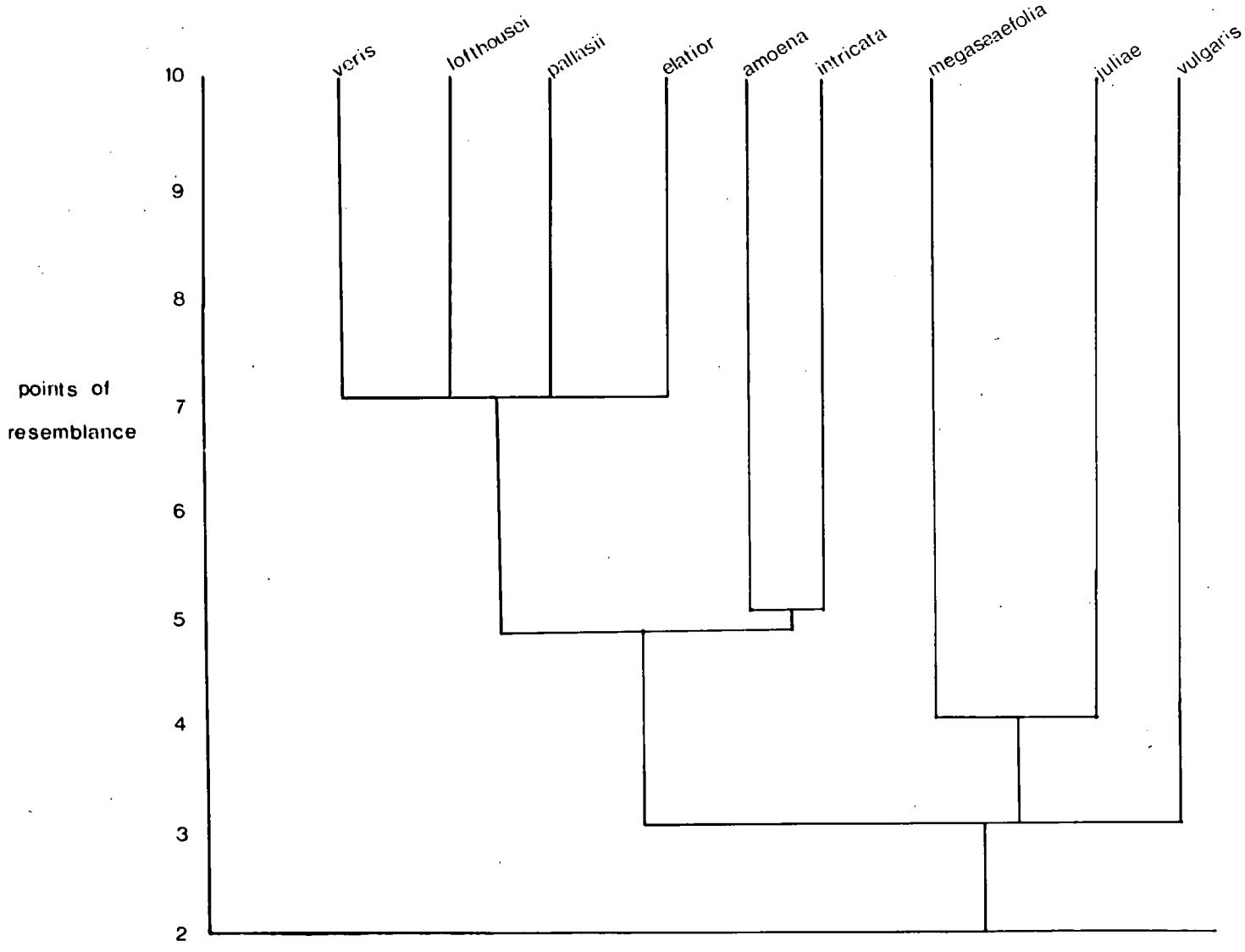


Figure 5 Dendrogram of the relationships of Primula species.

Cytology

The use of cytological observations is a common means of investigating the differences and similarities between taxa. As Swanson (1960) points out, "Cytology has become an accepted and exceedingly useful science in the hands of the taxonomist who was interested in something more than simple morphological criteria for defining species relationships. In fact, relationships within natural groups of species can hardly be considered to be complete in an evolutionary sense without good cytotaxonomic data to reinforce conclusions based on morphological criteria".

Mitosis

Cytology has already been used by Bruun (1932) to investigate relationships within the genus Primula as a whole. He found a good measure of agreement between the picture of relationships expressed by such features as chromosome number and morphology, and those expressed in terms of more conventional studies. Bruun established that in the Vernales section (now in Wendelbo's subgenus Primula), n = 11, and that this is probably the basic number for the whole genus. "As to size, the chromosomes are throughout small and short", Bruun notes, "with a length = 2-3 times their width. Furthermore, they are mostly

kidney-shaped or bent at an angle, indicating a median constriction". Bruun believed that changes in the nature of the chromosome complement may have occurred due to translocation. He believed that such a change involving "inversion or reversal of a segment" had occurred in a member of the section under consideration, P. leucophylla, (classified by Smith and Fletcher as a subspecies of P. elatior). He further believed that the change was associated with the satellited chromosomes, a pair of which are a feature of the karyotype of the group.

"On making a comparison with sections previously examined we find corresponding size of chromosomes and configurations only in Megaseaefolia. These two form a strictly delineated cytological type from the groups previously described. The conclusion is quite obvious that the Vernales and Megaseaefolia constitute a separate branch of the genus, so far removed from the other sections that no kinship with any other group can be demonstrated cytologically". Recent work of Valentine (1961) on seed incompatibility has suggested that megaseaefolia is more nearly associated with P. elatior than any other member of the group, while the numerical analysis presented earlier confirms the validity of associating it with the other members of the group.

Mitotic chromosomes

Primula appears to be difficult material in which to obtain satisfactory mitotic stages in root tips. Attempts were made to produce root-tip squashes in order to observe somatic chromosomes. Squashes were made after fixation in alcohol:glacial acetic acid:chloroform (3:1:1), both with and without pre-treatment. Colchicine, para dichlorobenzene, and cold treatment were all used as pre-treatment at different times. Unfortunately, no really satisfactory preparations were obtained, although the Feulgen technique, and aceto-carmine were used on material which had been stored in deep freeze in alcohol for some time, and acetic orcein was used on fresh material.

One difficulty was that not many cells appeared to be undergoing division, and those which were did not give satisfactory plates, for it was found difficult to spread the chromosomes.

Eventually, it was decided to follow Bruun's example and use sectioned material. Accordingly, root tips were fixed in Nawashin's fluid and embedded in paraffin wax. They were then sectioned at $12\frac{1}{2}$ mu thickness, and stained in crystal violet, being counterstained in orange G. Serial sections were then mounted in balsam. The chromosomes are apparently as Bruun described them in number and

configuration. However, it was not possible to add anything to Bruun's mitotic observations.

W. W. Smith (1933) commenting on the correlation of Bruun's cytological analysis of Primula with his own, more formal, taxonomic approach says, "Granted that there is a remarkable coincidence between the results of the two methods so far as the broader issues are concerned, will there be the same harmony when these investigations are carried into the field of greater detail?". Again, (ibid), "Too much cannot be read into grosser cytological phenomena. Approximation cytologically is not necessarily decisive as to close affinity. Until the cytologist can in this genus carry his analysis deeper, involving the qualitative content of the chromosomes and the different relationships between them, this difference of opinion will persist".

Smith & Levin (1967) write, "While distinctive karyotype differences almost certainly signal gross differences in the genetic makeup of the chromosome, it cannot be assumed that identical or nearly identical chromosome morphology is indicative of homology". The present study is an attempt to carry the cytological analysis of the species of the section Primula deeper, in an effort to discover and evaluate some of the different relationships between the chromosomes of the different

species of the section Primula, using the extent of pairing in interspecific hybrids, and the types of abnormality produced in interspecific hybrids.

Meiotic studies

Other than to suggest that the species might be closely related, since their chromosomes are so similar, the study of mitosis does not add a great deal to the picture of relationships within the group.

Davis and Heywood (1963), commenting on interspecific hybrids, make the point, "Frequent use is made of meiotic chromosome behaviour as a means of indicating relationships through the kind and degree of pairing which has taken place .....". Later, the same authors write, "Evidence from pairing at meiosis will give an indication of phyletic relationships", while Sokal (1963) writes, "Plant cytology more than any other field can lay claim to giving insights into the true relationships among organisms".

Many workers have made use of the extent and type of pairing in interspecific hybrids to draw conclusions about the relationships of the species concerned. Stebbins (1950) writes, "... the chromosomes of different species may look exactly alike as to size and form, but nevertheless, possess many differences in gross structure, such as translocations and inversions, which become evident only

when they pair with each other in species hybrids".

Stebbins goes on to say "..... the evidence indicates that the karyotypes of the original, unspecialized progenitors of most families of flowering plants were essentially symmetrical. Increased asymmetry of the karyotype, consisting of the evolution both of chromosomes with subterminal centromeres, and of inequality of size between the chromosomes of the same karyotypes has been a frequent, but far from universal, type of change accompanying increased specialisation in external morphology".

The extent of pairing and the formation of abnormalities may be used as an index of the relationships of the species, for, "closely related species are usually similar in these respects, and distantly related ones are often recognisably different ...", (Stebbins, 1950).

As Mentzel (1962) says, "During the evolution of species, chromosomes undergo changes which render them increasingly non-homologous with ancestral chromosomes and chromosomes of other species descended from the same ancestry". Thus, a consideration of the types and extent of these structural differences in the hybrids formed between the members of a group of species can "give an indication of phyletic relationships".

Among those who have used cytology to elucidate

relationships among species was Goodspeed (1954), who wrote, "Extent of chromosome homology as expressed by the amount and quality of pairing at M1 of F1 interspecific hybrids provides significant evidence concerning species origins and relationships in Nicotiana".

Löve and Löve (1960) go as far as to say, "Cytotaxonomy is the most effective tool for modern evolutionary classification of plants, and it is also the best method so far invented to study relationships between taxa at or above the species level".

A. Löve (1960) was even more forceful. "We must remember that the chromosomes determine the characters, whereas the characters do not determine the chromosomes". This extreme view is not accurate, for several workers, for example Riley (1960) and Jones and Rees (1964), have demonstrated genetic control of chromosome pairing. However, Riley (1966) states that although non-homologous regions have been observed to pair in some organisms, "such non-specific pairing is not followed by chiasma formation". "Not only is pairing confined to homologues but it is achieved with remarkable longitudinal specificity so that corresponding loci, or at least corresponding chromomers become juxtaposed".

Accordingly, it was felt that investigations into the

extent of pairing, and the nature of the configurations produced during meiosis in interspecific hybrids of Primula, might uncover differences in the compositions of the specific genomes which could be used to determine the evolutionary relationships of the species concerned.

Ideally, investigations into chromosome homologies and morphology during meiosis are carried out during prophase. At this time, duplications and inversions in the chromosomes of plants like maize are easily seen. As Stebbins (1950) points out, "In most species of plants, however, this stage cannot be easily observed", and this is certainly true of Primula. Consequently, deductions about the morphology of the chromosomes must be drawn from the subsequent behaviour of chromosomes during metaphase and anaphase. For example, Swietlinska (1963), has used the appearance of univalents and anaphase bridges and fragments to draw conclusions about the chromosomes of the Rumex species which were the parents of the hybrid which she was investigating.

Chromosomes during meiosis

Observations during meiosis in interspecific hybrids of this group of species have been made by Chittenden (1928), Huskins (1929) and Valentine (1952 and 1961). Chittenden found that the fertility of hybrids with P. juliae was quite

high, and that, "where reduction divisions of the pollen-mother-cells were studied they were found to be surprisingly regular for interspecific hybrids. Any irregularities that were found were the exception".

Valentine (1952) says of the F1 hybrid between P. elatior and P. veris, and between P. vulgaris and P. elatior: "It has however been possible to observe clearly the occurrence of multivalents, thus to observe the data of Table 8, (see Table 22), and to establish with some degree of certainty that in both the hybrids listed, the arrangement of eleven bivalents; ten bivalents and two univalents; nine bivalents, one trivalent and one univalent; nine bivalents and one quadrivalent do occur".

Table 22

(From Valentine (1952), Table 8): Pairing at M1 of meiosis in P.M.C.

Plant	Number of cells lacking multivalents and mostly 11 II or 10II 2I	No. of cells with 1 III	No. of cells with 1 IV
P. veris (family R5)	23	0	0
P. vulgaris x P. elatior (S24)	31	7	1
P. veris x P. elatior (C1)	22	12	4

From this it is clear that changes in the nature of the arrangement of the chromosomal material in the genomes of the different species had most probably taken place during their evolution, and that an investigation of the nature and extent of these changes might throw some light on the evolutionary relationships of the species concerned.

Technique

Buds were fixed at appropriate times in a mixture of chloroform:absolute alcohol:glacial acetic acid (1:3:1), to which a small amount of ferric acetate had been added as a mordant. If they were to be kept for any length of time, the buds were transferred to absolute alcohol and stored in deep-freeze until required. Squash preparations of anthers were made by staining with acetocarmine, and made permanent by mounting in Euparal after dehydration. Dehydration of earlier preparations was achieved by using a water:alcohol:series, but was later effected by freeze-drying followed by immersion in absolute alcohol.

Interpretation of observations

The theoretical basis for the study of meiosis in interspecific hybrids, depends upon the assumption that pairing takes place only between homologous parts of homologous chromosomes. The amount of pairing between the chromosomes of different parental sets can therefore be used as indication of their relationship, provided that

it can be shown that such pairing that does take place, will take place only between the chromosomes of the different parental sets, and not between chromosomes of the same set. In other words, it is necessary to show that autosyndesis does not occur. Autosyndesis may occur due to duplications in the chromosomes, or to the genetic control of pairing of non-homologous chromosomes, cf. Riley (1960) in wheat, and Chedda and Harlan (1962) in Bothriochloa. Whether or not autosyndesis does occur is best determined by examining meiosis in a haploid. "A study of haploid meiosis provides a clue to the nature of the whole chromosome complement", (Swanson, 1960).

Accordingly, an examination of pollen- mother-cells dividing in a haploid plant of P. veris was carried out.

Meiosis in haploid P. veris

Eleven cells in which the chromosomes were in a stage of maximum contraction were observed in haploid P. veris. In none of the cells was there any evidence of associations between the eleven chromosomes present. Each chromosome remained distinct from the other chromosomes, and there was nothing to indicate any association, let alone crossing-over, was taking place, (Plate 2).

This stage is apparently followed by a mitotic-like splitting of the chromosomes, with the orderly segregation

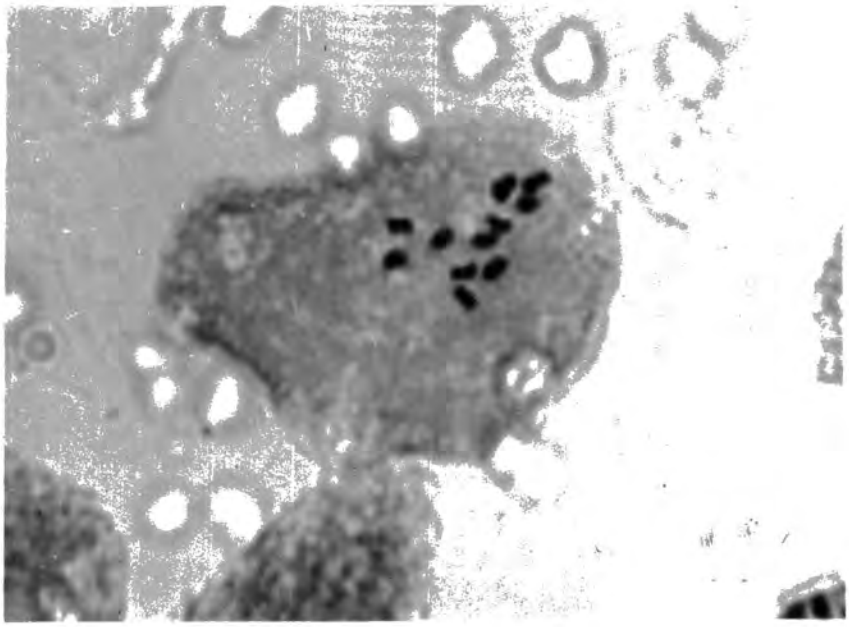


Plate 2. Division in a pollen-mother-cell of haploid P. veris, showing eleven univalents.

x1250

of the daughter chromosomes to opposite poles, each of which now has eleven chromosomes associated with it. No further division of the chromosomes takes place, and cytokinesis results in the formation of two quite normal looking pollen-mother-cells, instead of the four which are the products of meiosis in the diploid.

These observations are taken to support the view of Bruun (1932), that eleven is the basic number of the group under discussion, and that no extensive duplication of chromatin has occurred.

Meiosis in the diploid species

Observations on meiotic divisions have been made in the pollen-mother-cells of P. vulgaris, P. veris, P. lofthousei and P. elatior. Meiosis appeared to be quite normal in each case, eleven bivalents being formed. The bivalents consisted of rings, involving two arms of each chromosome, or rods involving only one arm (Plate 3). Disjunction proceeded in a normal fashion to produce ultimately four nuclei, each containing eleven chromosomes (Plate 4). Each of these nuclei was equal in size to the other three members of the tetrad. This is quite normal division.

A single plant of P. elatior proved to be exceptional in this respect. Although meiotic division appeared to

Plate 3. Early anaphase I in P. vulgaris. Chromosomes have been associated in eleven bivalents.

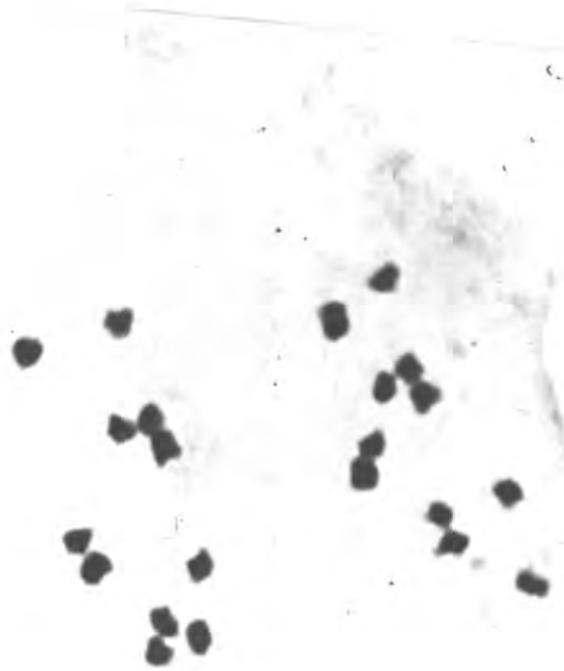


Plate 4. Anaphase I in P. vulgaris. Two groups, each of eleven chromosomes, showing that disjunction has been regular.

x1250

be regular in those cells in which it was studied, a small amount of chromosomal material was regularly left out of the tetrads to give extra nuclear bodies at this stage. This was apparently a regular event in this one plant, for which no explanation could be found.

However, the regularity of chromosome pairing, either eleven bivalents or ten bivalents and two univalents being found as a matter of course, shows that deviations from this regularity found in inter-specific hybrids must be taken as a sign of the lack of homology of their specific genomes, and interpreted accordingly.

Hybrid plants

Hybrids between several species of the section were available from Valentine's investigations into seed incompatibility in the group. They were produced by carefully controlled pollinations in an insect-proof greenhouse.

Chiasma frequencies in parental plants and F1 hybrids

The difficulty of obtaining prophase data in this material has already been mentioned. However, an attempt was made to assess the variation in pairing between certain of the species and hybrids under consideration, by estimating the number of chiasmata necessary to produce the metaphase configurations observed. This cannot give

an accurate picture of the chiasmata actually formed, since some chiasmata may have terminalised during prophase. However, it does give an indication of the differences between the species and the F1 hybrids in this respect.

Table 23 below contains data from the Primula observations, while Table 24 contains Darlington's (1937) data for chiasmata frequencies of some hybrids compared with their parents.

Table 23

Frequency of Chiasmata at M1 in some *Primula* species and hybrids

Plant	No. of cells investigated	Total chiasmata	Mean number of chiasmata per bivalent or pair of homologous chromosomes
P. vulgaris (B88b)	10	147	1.33
P. elatior (D1)	10	134	1.21
P. veris (C16)	10	142	1.29
F1: (*vulgaris* x *elatior*)(S24)	10	88	0.80
(*vulgaris* x *veris*)(A7k)	10	89	0.80
(*veris* x *elatior*)(C01)	10	89	0.80

Table 24 (from Darlington, 1937)

Chiasmata frequency of hybrids compared with their parents

Plant	Mean number of chiasmata per bivalent*
Triticum turgidum	2.43
T. durum	2.28
T. turgidum x T. durum	2.00
Kniphofia Nelsonii	1.8
K. Burchellii	1.6
K. Uvaria x K. Macowanii	1.4

*(Darlington records some "bivalents" with no chiasmata)

It can be seen that the Primula data shows a characteristic reduction of chiasmata frequency in the F1 hybrids, showing that there is some reduction in homology between the two specific genomes.

Meiosis in F1 hybrids

(a) (P. vulgaris x P. elatior)

In this hybrid several abnormalities of division have been observed, and they will be described here. However, a number of cells were seen in which eleven bivalents were

to be plainly seen, showing that the chromosomes from the one specific genome were pairing with their homologue from the other set. This is taken to indicate that the two genomes have a great deal in common with one another. In this hybrid, as in all the others, ten bivalents and two univalents were observed, but in nearly all cases the univalents were in close proximity to one another, and may have merely slipped apart after early terminalisation of chiasmata. In any case, the appearance of ten bivalents and two univalents has also been observed in the species, so that this condition cannot be regarded as abnormal.

However, the appearance of more than two univalents at metaphase one can be taken to indicate a reduction of homology between the specific genomes concerned, resulting in a reduction in chiasma frequency. Such reduction in homology is often the result of many small differences between the chromosomes concerned, and not to major differences, which can be expected to manifest themselves in other ways.

There is plenty of confirmation of Valentine's observations of multivalents in this hybrid. These are due to translocations which have occurred between chromosomes of one specific set, so that one species will have chromosomal material on one chromosome homologous with

that on two chromosomes in the other genome. Translocations are common in a number of plant species, where they are recognised by the presence of rings or chains of chromosomes in individuals heterozygous for them, (Stebbins, 1950). A hybrid heterozygous for a translocation may thus produce chromosome configurations as follows:- two bivalents; one trivalent plus one univalent; one quadrivalent; four univalents. In the F1 (P. vulgaris x P. elatior) all of Stebbin's possible configurations are without doubt achieved. Certainly the cells containing eleven bivalents represent his first case in an individual which is heterozygous for a translocation, as this one is. Cells with either a quadrivalent (Plate 5) or a trivalent plus a univalent (Plate 6) are found. The possibility that the univalents found in cells with nine bivalents and four univalents are due to interference with pairing due to this structural difference between the chromosomes is a strong one. Some cells in this hybrid contain two quadrivalents, or two trivalents and two univalents, demonstrating that more than one translocation has occurred to differentiate the two specific genomes concerned.

Univalents tend to remain on the equator, and then divide into two at first anaphase, which will result in a failure to divide again at second anaphase, and the

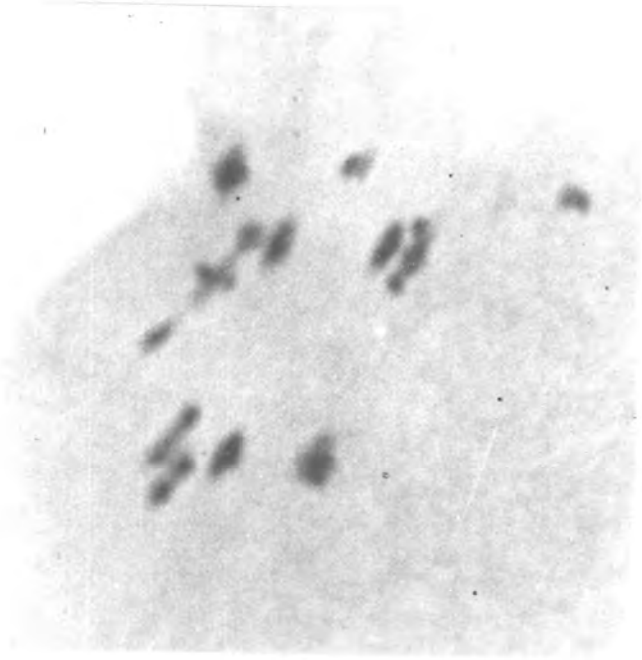


Plate 5. Metaphase I, in F1 (P. vulgaris x P. elatior). Plate shows one quadrivalent, eight bivalents, and two univalents.

x1250

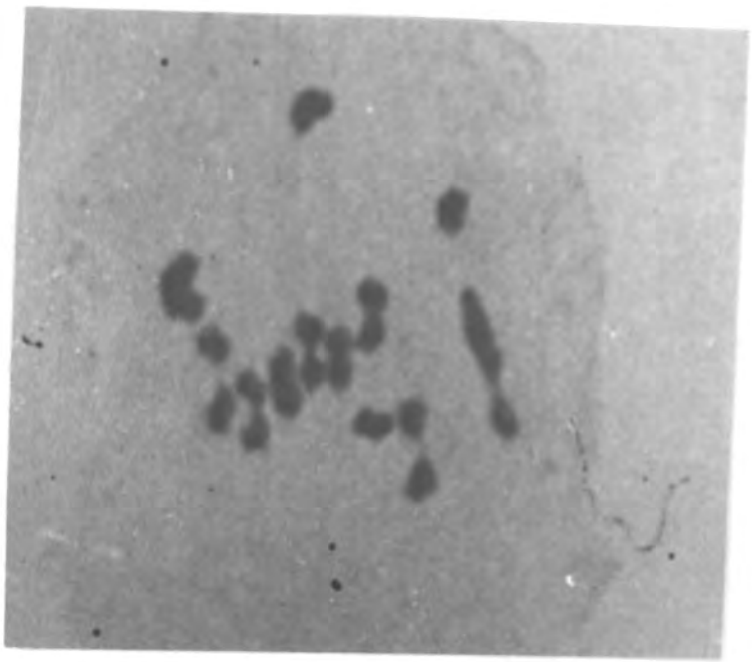


Plate 6. Metaphase I, in F1 (P. vulgaris x P. elatior). Plate shows one trivalent, eight bivalents, and three univalents.

×1250

consequent distribution of chromosomal material in an unequal fashion to the four haploid nuclei formed as a result of the division. Darlington (1937) notes that unpaired chromosomes may vary from the meiotic to the mitotic mode of behaviour during meiosis, that is they may divide at once as appears to be the case in the Primula hybrids (see Plate 7), or they may pass entirely to one or other pole. Peto (1934) suggests that only those univalents lying close to the metaphase plate actually divide at first anaphase. The others are included in one or other nucleus in their entirety, and divide normally in the subsequent division. My observations do not contradict this view.

As a result of chiasma formation between chromosomes which are only partially homologous, the terminalisation of chiasmata may be interfered with, resulting in the non-disjunction of chromosomes at anaphase. "Assumption must be made that at some stage terminalisation is suspended so that if it is not complete the chiasma will not have reached the ends of the arms. The arrest is probably brought about by the degree of contraction of the chromosomes, the two functions not being coordinated", (Darlington, 1931). This non-disjunction may produce one of two manifestations of translocations at this stage of the division. On the

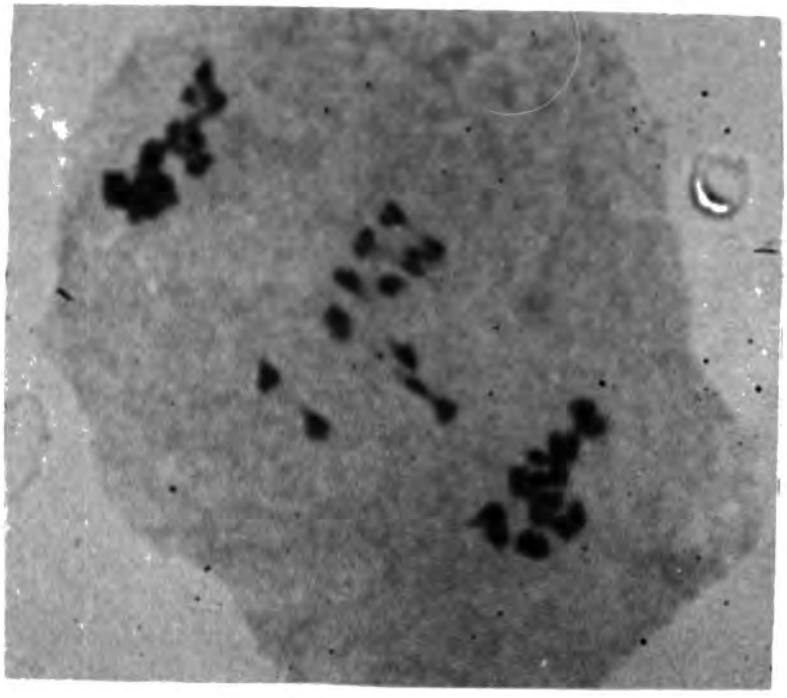


Plate 7. Late anaphase I, in F1 (P. vulgaris x P. juliae). Dividing univalents at late anaphase I. At each pole are eight chromosomes. They presumably paired and disjoined normally. The six chromosomes left as univalents now dividing precociously. x1250

one hand, the two chromosomes being unable to separate properly may both proceed to the same pole, resulting in an unbalanced segregation such as 10 + 12, instead of 11 + 11, which would be expected normally (see Plate 8). If the still paired chromosomes do proceed to opposite poles the result may be a chromatin bridge at anaphase, again possibly resulting in mis-division, since some of the genetic material will be left out of the daughter nuclei after cytokinesis, (see Plate 12).

It is thought that intra-chromosomal translocations or inversions, may be recognised in this hybrid by the appearance of anaphase bridges and associated fragments. These have probably been formed by the formation of chiasmata in the inverted segment which is necessary for pairing between homologous parts of chromosomes which differ from one another by an inversion. As a result of this, disjunction produces a single piece of chromatin with a centromere at each end, and a piece of chromatin without a centromere. At anaphase the centromeres separate, stretching their chromatin connection between them as a bridge, and leaving the chromatin without a centromere on the metaphase plate as a fragment, (Plate 9). Stebbins (1950) says, "Inversions of chromosome segments are likewise well known and probably occur in an even greater number of

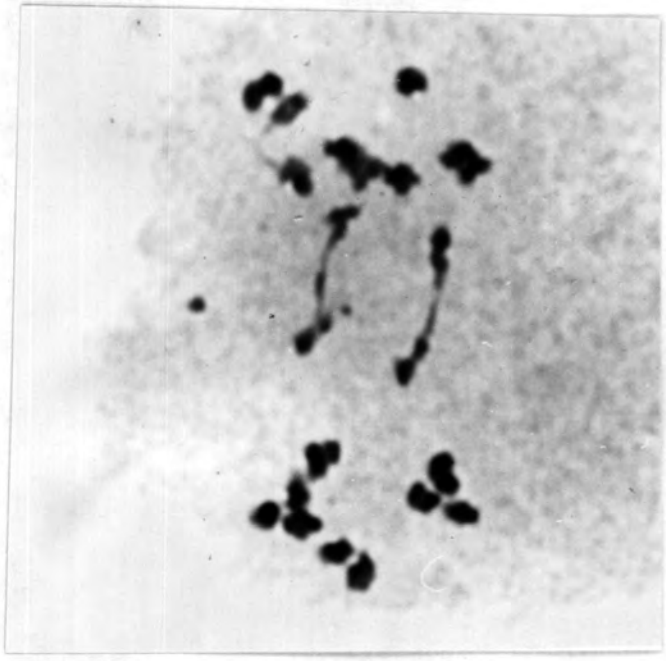


Plate 8. Anaphase I, in F1 (*P. vulgaris* x *P. juliae*). Two anaphase bridges, one due to non-disjunction, the other to inversion, are seen. In addition, this illustration shows unequal segregation of chromosomes, 10 + 12 instead of 11+ 11, again probably due to non-disjunction. x1250

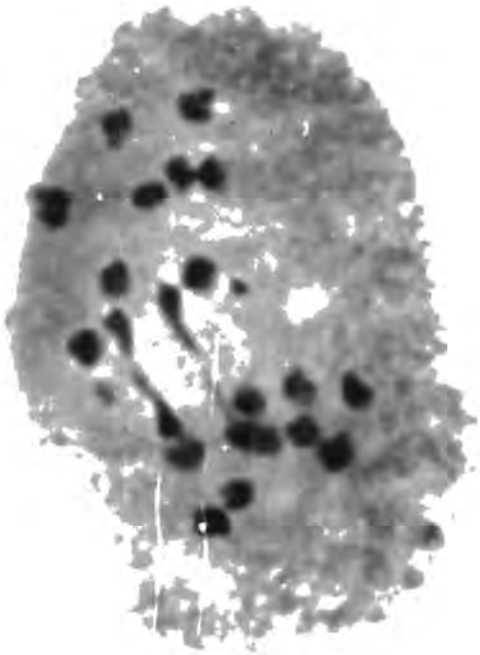


Plate 9. Late anaphase, in F1 (*P. vulgaris* x *P. elatior*). Two groups of eleven chromosomes. Two pairs of homologous chromosomes at each pole are still connected by inversion bridges. Each bridge has an associated fragment.

x1250

plant species than do translocations", and Lewis and John (1963) says, "the commonest type of intrachromosomal translocation is the inversion".

However, Newman (1966), after investigating bridge and fragment formation in Podophyllum, has echoed the doubts of other workers such as Matsuura (1950), Haga (1953) and Walters (1950). These workers question the validity of accepting bridges and fragments at anaphase as evidence of inversion, and Newman has shown that anaphase bridges and fragments in Podophyllum are not necessarily associated with the characteristic pachytene foldback, in other words, they are not due to inversions, but to some other cause. However, when the bridges and fragments occur as they do in this investigation in F1 hybrids between different species, and there is no evidence of their occurrence in the pure species, then their occurrence due to differences between the two specific genomes, that is inversions, seems the more likely cause. Further support for this view is that in a hybrid the fragments tend to be of the same size, and are so presumably due to something a little more regular than a haphazard fragmentation.

However, caution must be exercised in accepting bridges and fragments at anaphase as absolute proof of inversions, so that the presence of such differences can only be said

to be a strong possibility in the absence of confirmatory prophase data.

Again, bridges formed in this way may interfere with cytokinesis. On the other hand, in the case of both inversions and translocation, the bridges may be snapped, resulting in the formation of cells which are unbalanced chromosomally and genetically.

It is also likely that non-disjunction will result in the leaving of bivalents on the metaphase plate after the other chromosomes have reached the poles, with the result that these whole chromosomes will not be contained inside the tetrads, but will appear as separate, extra staining bodies at this stage of division.

As a result of the abnormalities of division which occur during meiosis in the hybrids, the form of the tetrads may be modified. Instead of each of the daughter nuclei possessing a complete haploid set of chromosomes, they may contain more or less than this number. As already explained, cytokinesis may be interfered with, due to the occurrence of bridges, or to lagging bivalents and univalents. The result will be the formation of a cell containing a single restitution nucleus, containing anything from the diploid to the tetraploid number of chromosomes. Alternatively, the same mechanism may result in the formation

of two cells instead of four.

Other tetrads are formed with different chromosome numbers, indicating that an irregular distribution of chromosomes has taken place, with the incorporation of more than the haploid number of chromosomes into one nucleus, again due to the reasons already put forward.

Similar reasons may also account for the appearance of more than four nuclear bodies at this stage, for the univalents and bivalents may fail to leave the metaphase plate and be left out of the main nuclei, remaining as extra nuclear material, see Plate 12.

Following the production of abnormal tetrads due to abnormalities at meiosis, it is inevitable that the pollen grains themselves should reflect some of this abnormality (Plate 13), and this itself could be used as an index of the relationships of the species forming the hybrid.

The absence of chromosomes from the pollen grains will mean that some of them will be smaller, others, with extra chromosomes, will be larger than the normal grain. This can be particularly noticed when they have been stained with acetocarmine. "Grains were considered to be normal and presumably viable if they were found, fully stained and if the nuclei had a normal appearance", (Dunford, 1964).

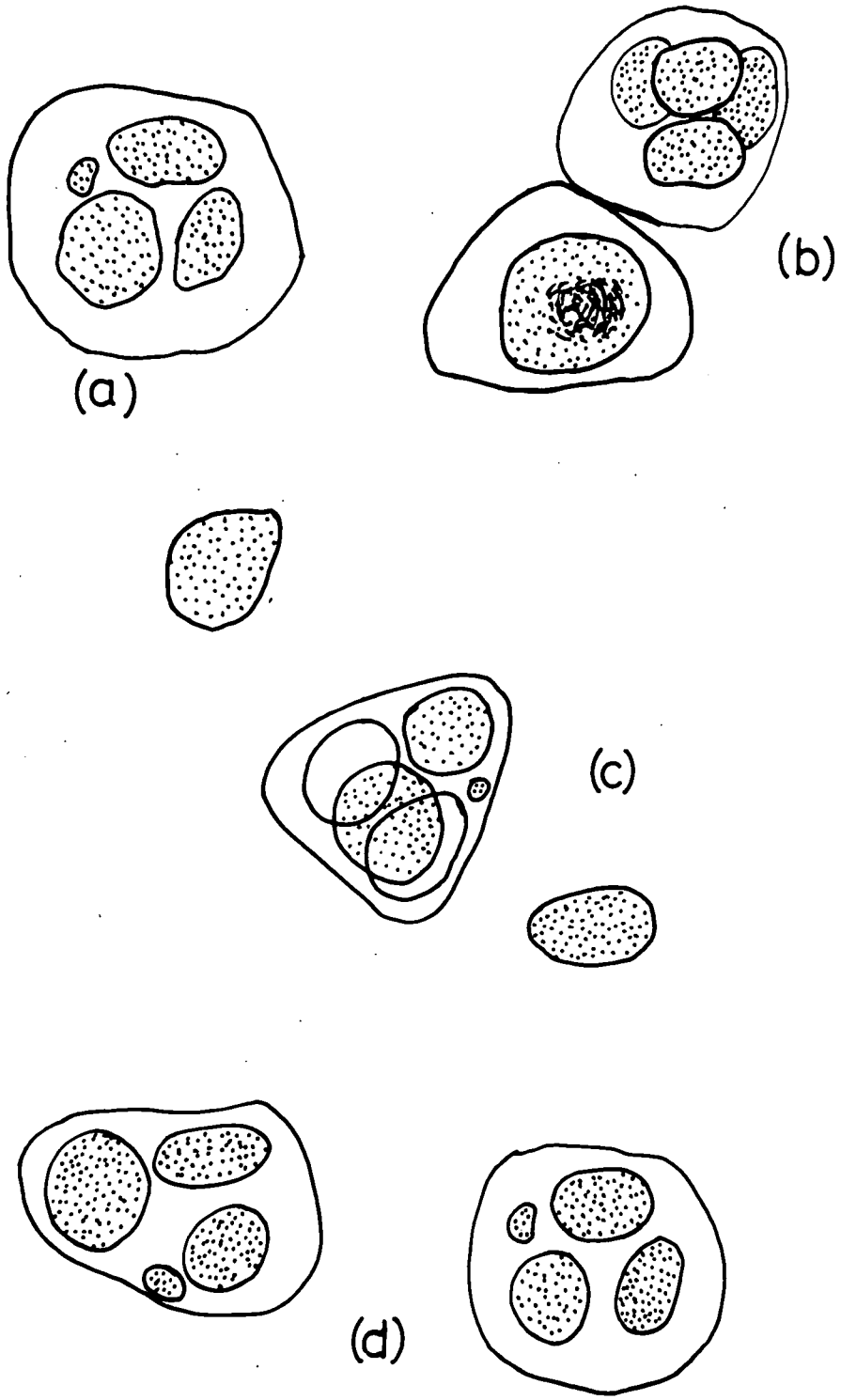


Plate 10. Types of "tetrad" seen in F1 diploid hybrids.

The upper tetrad in (b) is apparently normal, the lower shows restitution after abnormal meiosis.

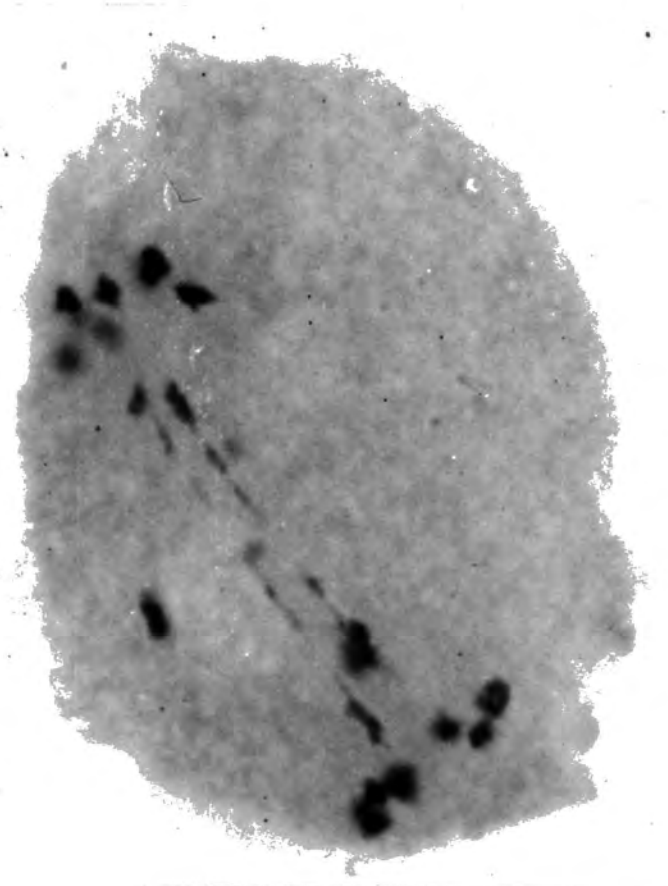


Plate 11. Inversion bridges and fragments in F1 (*veris* x *vulgaris*).

x 1250

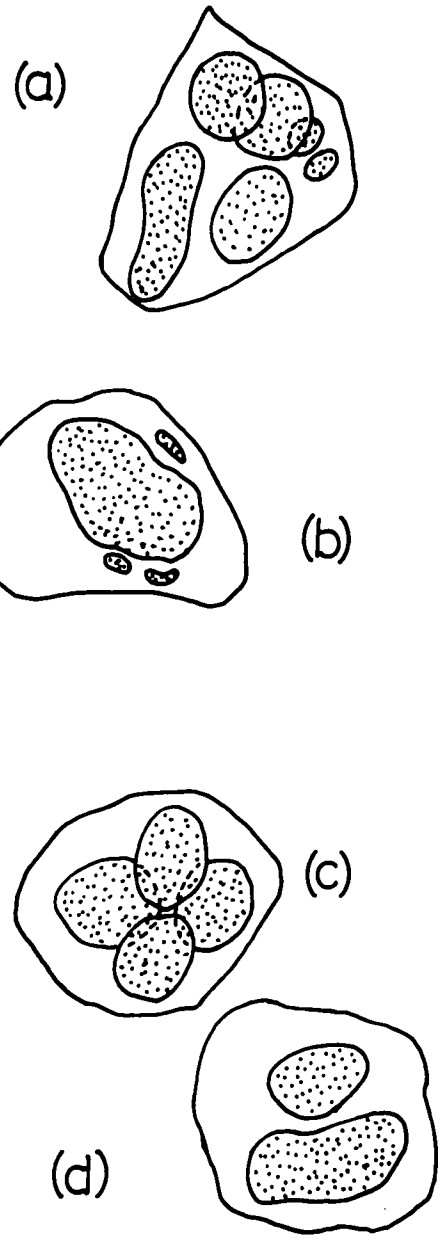


Plate 12. Abnormal tetrads in F1 tetraploid hybrids

(c) shows a normal tetrad, while (a), (b) and (d) show some of the abnormalities.

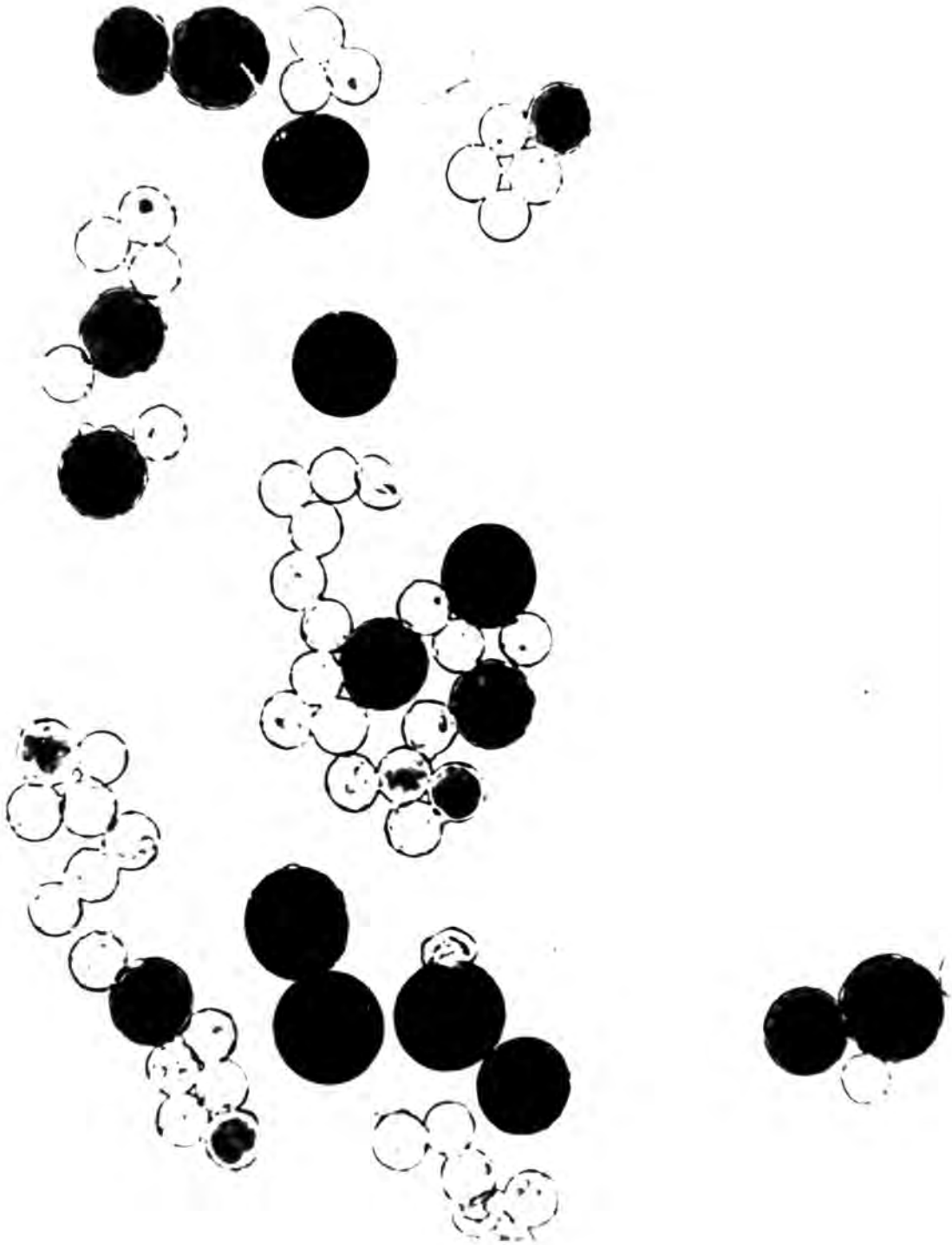


Plate 13. Abnormal pollen in hybrid.

Meiosis in F1 hybrids: (b) Other hybrids

Appendix E presents the results of the observations on meiosis in interspecific hybrids. From this it will be seen that all of the hybrids are characterised by one or more translocations. Even the hybrid (P. pallasii x P. megaseaefolia), where there is no evidence of translocations at metaphase, possesses evidence of this difference in the form of non-disjunction bridges at anaphase one. The greatest amount of translocation is seen in the hybrid F1 (veris x vulgaris, see Plate 11), where 42.4% of the cells at metaphase one contain evidence of one or more translocations, the specific genomes concerned differing from one another by a minimum of three such translocations.

Table 25 shows a comparison of the percentage of normal cells seen at metaphase one with the percentage of normal pollen produced, and Fig. 5b shows these data compared in graph form.

Table 25

Correlation of percentage of normal cells at M1 and normal pollen

Cross	% normal cells at M1	% normal pollen
(P. veris x P. vulgaris)	40.6	32
(P. veris x P. elatior)	68.3	43
(P. vulgaris x P. elatior)	79.0	75
(P. juliae x P. vulgaris)	89.3	60

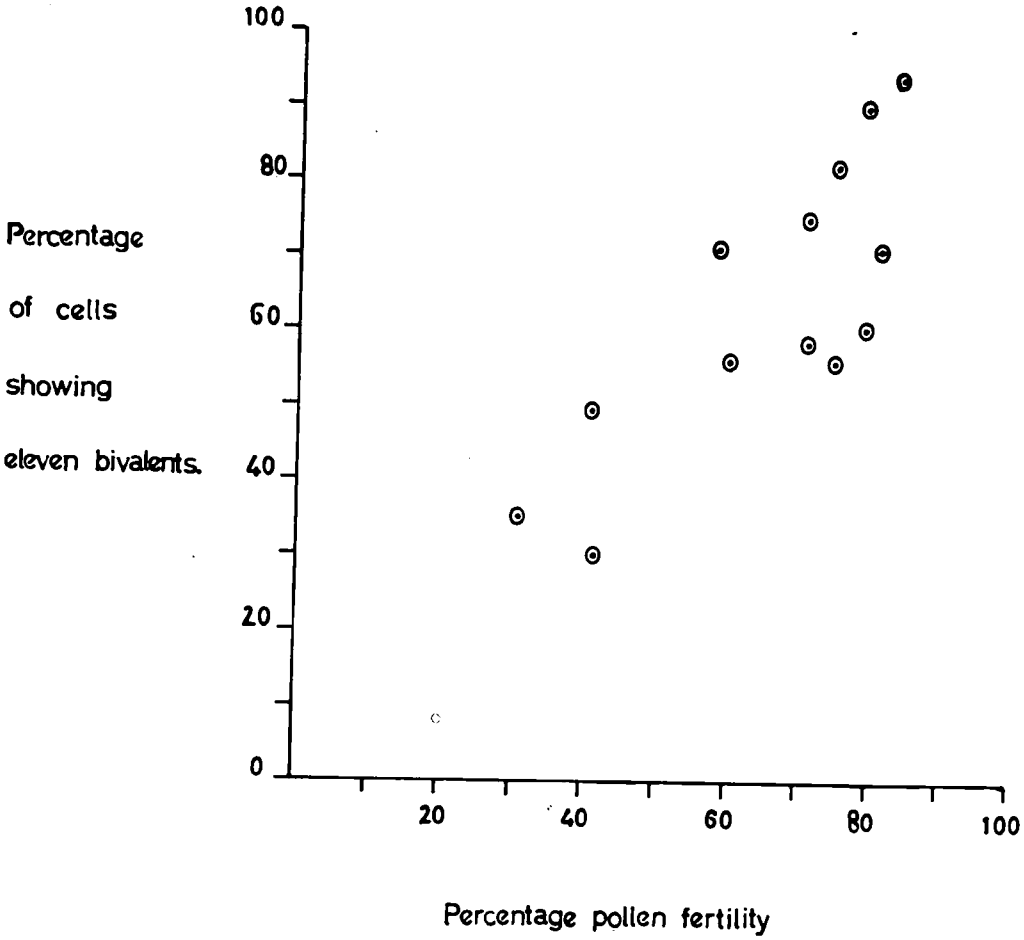


Figure 5b Relationship of cells showing normal division at meiotic metaphase in interspecific F1 hybrids, to sound pollen.

In general terms there is a broad correlation between the two, with the hybrids which produce the greater numbers of abnormalities at Metaphase, (P. veris x P. vulgaris) and (P. veris x P. elatior), producing the least amount of good pollen.

Where discrepancies do occur between the amount of normal division and the amount of good pollen, this is in the right direction. In other words there is more bad pollen than could be accounted for by metaphase abnormalities, and this can be explained by the effects of irregularities like inversions, which do not manifest themselves until anaphase, but which will nevertheless interfere with the formation of viable pollen.

The greatest amount of meiotic abnormality and abnormal pollen is seen in the hybrid (P. veris x P. vulgaris), suggesting that these two species are the most distantly related of the group. P. elatior is the next most distant relative of P. vulgaris, while this evidence seems to indicate that P. juliae is the latter's closest relative.

Taking the relationships of P. juliae, then its closest affinity is with P. elatior, with P. vulgaris next, and P. veris very much more distant.

Thus, the picture emerges of a group of three fairly closely related species, P. vulgaris, P. juliae and P. elatior, forming a group of species only more distantly related to

P. veris.

Of the other species of the section, P. lofthousei, P. intricata and P. pallasii have been variously assessed. Smith and Fletcher (1947) include all three as sub-species of P. elatior, along with P. elatior itself. Hybrids between P. elatior and P. lofthousei, and P. elatior and P. intricata have been analysed, with the results presented in Appendix E. In both of these taxa there is evidence of translocations separating them from P. elatior proper. In the case of P. intricata one translocation has been observed, and 91% of the metaphase cells are apparently normal. With P. lofthousei, on the other hand, there is evidence for two translocations, and only 85% of the metaphase cells are normal. This would indicate that P. intricata is more closely related to P. elatior than is P. lofthousei, and this agrees with the relationships expressedby the seed incompatibility data, (Valentine 1961).

No data is available for the cross (P. elatior x P. pallasii), but the latter species differs only slightly from the genome of P. megaseaefolia, which again differs only slightly from P. elatior. Similarly, P. amoena differs only slightly from P. lofthousei.

The data shows that the taxa are therefore very closely related indeed. The actual relationships, and

the significance of this ranking will be dealt with later.

Another of the features of Grant's table of factors differentiating species may be added to the list of those found differentiating Primulas. His fifth category, that of chromosomal rearrangements (see Table 3, page 12), can be added to the ways by which Primula species differ from one another.

A similar study to the investigations of Primula relationships on the basis of cytological evidence has been reported by Levin (1966). His investigations "have proved enlightening with respect to patterns of relationships in the Phlox pilosa complex".

There are several parallels between the Primula and Phlox situations. Both are outbreeders, and the incompatibility barriers between species are readily breached. The Phlox complex consists of twelve morphologically distinct taxa which have been incorporated in four species by Wherry (1955).

Levin measured chromosome homology in hybrids by their pairing relationships and the frequency of chiasmata. Interchanges were recognised by the appearance of quadrivalents and trivalents at metaphase, and the greatest amount of pollen sterility, estimated by staining the pollen with aniline-blue-lactophenol solution, occurred in

interchange hybrids. Lagging chromosomes at anaphase I and II were associated with a reduction in chiasmata frequency. Cryptic structural hybridity, revealed in hybrids by precocious desynapsis of bivalents, and reduced chiasma frequency, has proceeded more rapidly than the major repatterning of chromosomes caused by inversions or translocations.

As a result of his cytological studies, Levin has been able to construct a picture of relationships of taxa within the complex. "Impressions of discontinuity based upon morphological considerations are, in most instances, strengthened by the experimental data". The Phlox investigation is one in which the study of chromosomes in hybrids between taxa has added to an understanding of their relationships in a confused situation. To some extent the information from similar investigations does likewise.

Polyploids

An investigation of the relationships of the genomes of different species cannot be considered to be complete without an analysis of allopolyploids. Stephens (1943) has stated that in such studies allotetraploids give a better indication of affinities than do the diploid hybrids. In the latter in the absence of completely homologous

partners, relatively slight affinity between chromosomes may lead to pairing. In the allotetraploid where every chromosome has an exact homologue, any "overpairing", that is the formation of polyvalents, must indicate a close affinity between the chromosomes of the genomes concerned.

A good example of the case where the polyploid reveals the true situation in a hybrid is that of Primula kewensis, reported by Newton and Pellew (1929). Here the diploid hybrid between P. verticillata and P. floribunda is completely infertile although "nine loosely paired bivalents are formed, and there is as a rule no irregularity, nine chromosomes being segregated to each of the spores of the tetrad. ..... we have not observed any more fertile plant showing more regularity in meiotic behaviour".

In fact, the two specific genomes differ from one another sufficiently to bring about infertility in the F1 due to segregation producing gametes containing a mixture of the chromosomes from the two species.

The tetraploid hybrid is fertile however, usually showing sixteen bivalents and one quadrivalent at metaphase. This shows that in the presence of an identical homologue from the same specific genome, a chromosome from one species will not generally pair with a chromosome from another, since their degree of similarity is not sufficiently great.

Thus, the difference between the genomes is revealed as being greater than might be expected from the diploid results alone.

With this in mind investigations were made into meiosis in the allotetraploids 4n (P. vulgaris x P. veris) and 4n (P. vulgaris x P. elatior), and the allotriploid (2n P. veris x 4n P. elatior).

In order to assist with the interpretation of the results derived from these observations, observations of pollen-mother-cell divisions in autotetraploid material of P. elatior and P. veris were accumulated and analysed.

Meiosis in autotetraploids

If there is a maximum association between homologues, one might expect to find eleven quadrivalents present in the Primula material. However, as Darlington (1937) points out, "recent work has shown that, while maximum association does occur in most such forms in a proportion of nuclei, in a proportion it also fails. Trivalents are replaced by bivalents and univalents in the triploids and by pairs of bivalents in the tetraploids. Association is therefore incomplete and variable .....".

He presents a table to illustrate this variation (see Table 26).

Table 26

(Table 15 from Darlington, 1937)

Variation of pairing in triploids and tetraploids

	Numbers of cells with different numbers of trivalents												
Triploids 3x = 36	0	1	2	3	4	5	6	7	8	9	10	11	12
Lilium tigrum							2	4	9	25	14	12	4
Tulipa gesneriana								1	4	8	5	-	1
Pink beauty						2	1	3	2	6	5	2	3
Inglescombe yellow						1	-	1	2	1	2		
Solanum lycopersicum				5	13	17	10	5					

	Numbers of cells with different numbers of quadrivalents												
Tetraploids 4x = 48	0	1	2	3	4	5	6	7	8	9	10	11	12
Solanum lycopersicum		3	12	10	2	15	6	0	2				
Primula sinensis										1	11	9	

Table 27

Numbers of polyvalents in cells of autotetraploid Primulas

	Number of cells with different numbers of polyvalents											Total cells
2x = 44	0	1	2	3	4	5	6	7	8	9	10	11	
P. veris (C16)	2	3	1	4	4	11	9	5	5				44
P. elatior (G238)	1	1	4	3	8	4	1	0	1				23

C16 was made tetraploid by means of colchicine treatment, while G238 was produced by crossing two already tetraploid plants.

The same sort of variation in the number of polyvalents formed in autotetraploids is seen in the data for Primula obtained during the present work and set out in Table 27.

The average number of polyvalents in each metaphase cell of P. veris is 4.7, and in P. elatior 3.6. The calculations of chiasmata frequencies in the diploid species presented in Table 23 gave P. elatior a lower frequency of chiasmata (1.12), than either P. vulgaris (1.33) or P. veris (1.29), so that the decreased chromosome association is what one would expect if this depends on chiasmata frequency.

Darlington (1932 and 1937) argues that polyvalent frequency depends upon the effective length of chromosome available for pairing, and upon the "frequency of chiasmata formation between chromatids at diplotene". More recently Morrison and Rajhathy (1960a) have expressed doubt about the relationship between chiasmata and polyvalent frequency. They found that a "study of ten 4n plants representing different families and orders of plants with small and large chromosomes support the hypothesis that in all autotetraploids approximately two-thirds of the chromosomes form quadrivalents". Later (ibid) they say: "Our results then, show no evidence of gene control over quadrivalent frequency and support the hypothesis that the behaviour is the same in all species". The same authors state (1960b) that, "chiasmata frequency of the autotetraploids was less than twice that of the diploids. No definite correlation

relating chiasmata frequency to the number of quadrivalents could be established".

Roseweir and Rees (1962), do not support this point of view, however, and they write: "(1) Theoretically, the types and frequencies of the associations, IV; III + I; II + 21 and 41, must depend partly upon chiasmata frequency. (2) Chiasmata frequencies are genotypically controlled.

Both of these factors are relevant and operate to control the fertility of autotetraploid rye:

(a) segregation of genes for chiasma frequency.

(b) when the frequency of quadrivalents and other configurations are plotted against chiasma frequency of the F2 plants, it is seen that the distribution of the various chromosomal configurations are dependent upon chiasma frequencies".

Pearson (1965) has prepared two models of chromosome behav iour in autotetraploids, making the assumptions noted below. The information was fed into a computer, which gave the percentages of configurations to be expected.

Pearson's two models were produced on the following assumptions, with the results indicated.

Model ONE.

Assumptions made: 1. Chromosomes all equal in length.
2. Centromere median.

3. Each arm has equal chance of pairing, with a frequency of one chiasma per chromosome.

4. Pairing is completely at random.

Results:	Quadrivalents:	53.5% of the total number of chromosomes.
	Trivalents:	6.2% "
	Bivalents:	43.0% "
	Univalents:	6.4% "

Model TWO.

Assumptions made: 1.) 2.) 3.) As for model ONE.

4. Chiasmata are inserted sequentially.

Results:	Quadrivalents:	44.0% of the total number of chromosomes.
	Trivalents:	Completely eliminated by point 4.
	Bivalents:	46.2% of the total number of chromosomes.
	Univalents:	5.0% "

The assumption made in model 1 are straightforward and self explanatory. However, point 4 in model 2, "chiasmata are inserted sequentially", perhaps requires some further explanation.

Sequential insertion means that each and every pair of chromosomes receives one chiasma, so that every pair of chromosomes possesses at least one chiasma, before any further chiasmata are distributed. In other words, one pair of chromosomes could not receive two chiasmata while

another pair of chromosomes was without any.

This kind of control of chiasmata distribution, as well as frequency, is well known in some plants, vide Jones and Rees (1964). They observe that not only the mean chiasma frequency may be under genetic control, but also the distribution of chiasmata between cells. By referring to Rees and Thompson (1956), they draw attention to evidence that suggests that the two characters may vary independently.

Rees and Jones point out that factors which affect the chiasma frequency of bivalents within cells are known to be, for example, the variation in chromosome length (Mather, 1938), and changes in chromosome structure (Jain and Bose, 1960). In addition, their own work confirms that, "over and above these structural factors, the genotype exercises considerable control upon the distribution of chiasmata between bivalents".

"It is debatable whether completely random chiasma formation ever occurs, either in plant or animal meiosis", writes Henderson (1961). He believes that localisation of chiasmata is the rule, rather than the exception.

Riley (1966) states: "It can therefore be concluded that all the events of meiosis are under some form of genetic control".

Autotetraploid P. elatior and P. veris

The results of examining meiosis in pollen-mother-cells of autotetraploids of P. elatior and P. veris are presented in Appendix F.

It is of interest to note that in both of the auto-tetraploids, despite the fact that there are four homologues of each chromosome present, there are some cells in which only bivalents are formed. These will undoubtedly give rise to the cells at anaphase one which contains two groups of chromosomes at the poles, each group containing twenty two chromosomes. Some of these normal anaphase cells will be the result of the regular disjunction of chromosomes which were associated in higher configurations, for as Darlington (1931) has pointed out, there is a chance that chromosomes associated in quadrivalents will undergo normal disjunction. That there need be no further interference with the stages of division is shown by the appearance at anaphase two of four groups of chromosomes, each group containing twenty two chromosomes, and by the formation of normal looking pollen grains.

Autotetraploid P. elatior has been successfully used by Valentine as the pollen parent in a cross with diploid P. veris, giving a hybrid with thirty three chromosomes, indicating beyond doubt that regular division does produce

pollen with twenty two chromosomes.

More typically in the autotetraploids, however, higher associations of chromosomes are found in 95% of the cells examined. In no cell has a greater number of polyvalents than eight been seen (see Fig. 7), and most have considerably fewer than this. This follows the pattern for behaviour in the autotetraploid to be expected after referring to Darlington's data presented in Table 2[illegible].

The most common arrangement of chromosomes in a quadrivalent would appear to be a chain of four, although a few rings of four have been seen. Trivalents with univalents have also been seen, and these are illustrated in Fig. 6.

Although regular division of chromosomes associated as polyvalents may occur at anaphase, the possibility of non-disjunction exists, with the result that unbalanced segregations can give rise to such groups as (21 + 23) and (20 + 24), which have been seen at anaphase one.

Comparison of the Primula data with Pearson's models

The sequential insertion of chiasmata required for Pearson's second model can be ruled out at once. Such a requirement means that no trivalents would be formed, while trivalents are in fact found in both of the autotetraploids examined. The percentage of chromosomes found in the

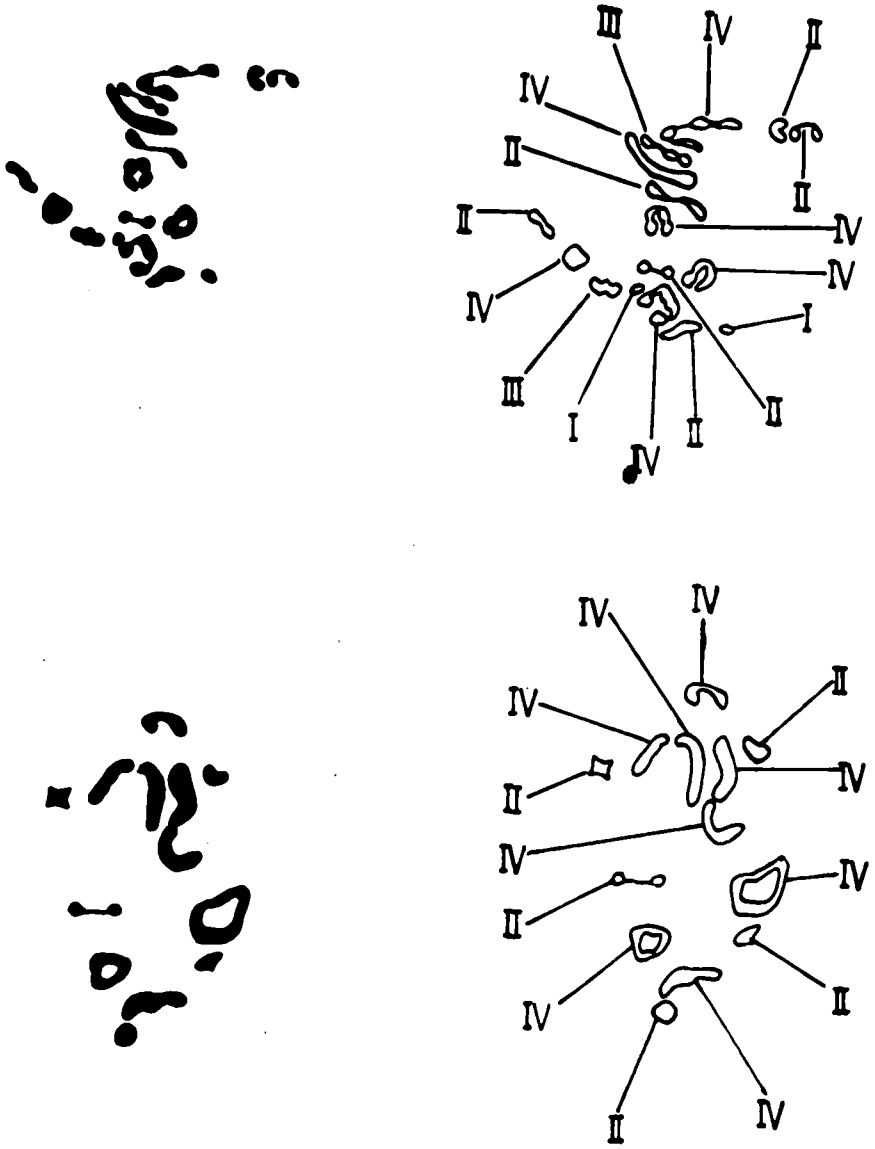


Figure 6. Meiosis in autotetraploids.

Camera lucida drawings of typical metaphase 1 configurations.

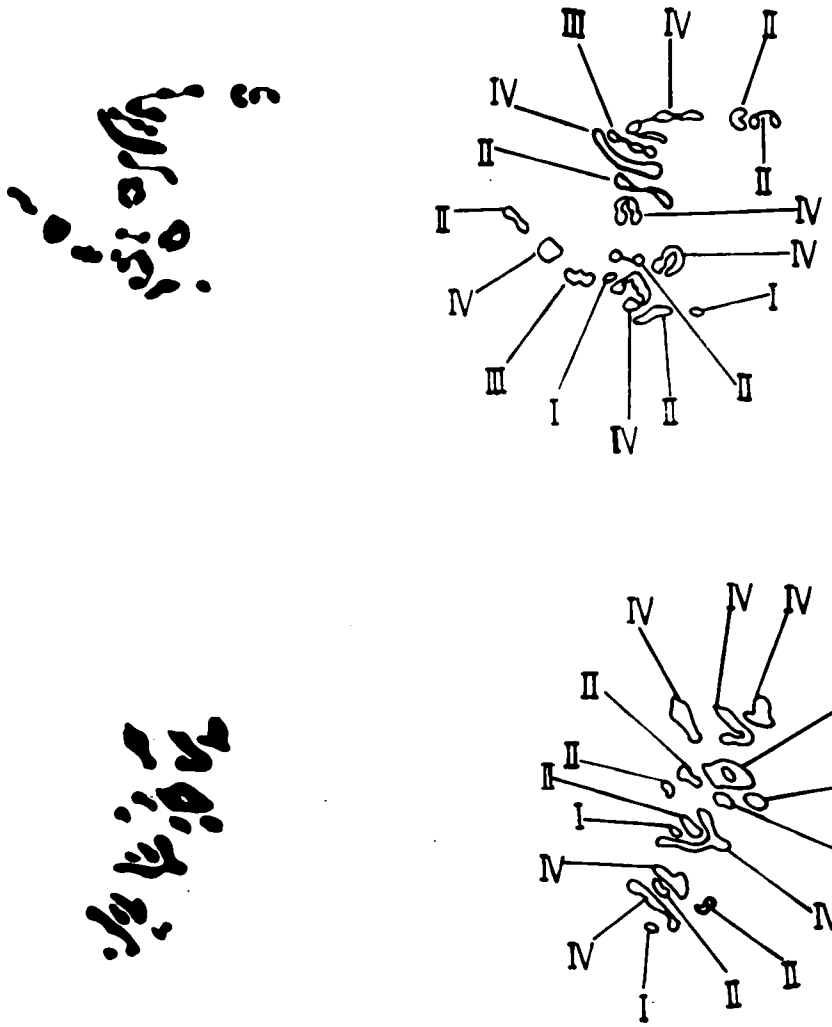


Figure 7. Meiosis in autotetraploids.

Camera lucida drawings of typical metaphase 1 configurations.

various types of configuration in autotetraploid P. elatior and P. veris are shown in Table 28.

Table 28

Percentages of chromosomes in various configurations in autotetraploids

Plant	Quadri-valents	Triva-lents	Biva-lents	Univalents
C16 4n P. veris (45 cells)	37% (740)	5% (108)	54% (1070)	3% (62) (= 99.0%)
G238 4n P. elatior (23 cells)	26% (264)	5% (51)	64% (656)	4% (41) (= 99.0%)
Expected (Model A)	53.5%	6.2%	43.0%	6.4% (= 109.1%)

The actual numbers of chromosomes in each category are in brackets after the percentage figure in Table 28.

Heterogeneity X^2:

C16: X^2 = 11.496 D.F. = 3; ∴ P of agreeing with model = less than 0.001.

G238: X^2 = 16.31; D.F. = 3; ∴ P of agreeing with model = less than 0.001.

In each case the results are significantly different from those calculated by the computer on the assumptions stated, which include the possibility of chiasma formation between each pair of chromosome arms. In both cases here there is a deficiency of polyvalents, which argues a lower

frequency of chiasmata than that assumed in the model. When it is borne in mind that even the diploid species exhibit a lower frequency of chiasmata than that assumed in Pearson's model, cf. page 96, then the results presented here are quite as expected.

Anaphase data. A few observations of anaphase one and anaphase two data in autotetraploid cowslip are available. Of the fifteen cells at anaphase one, ten showed apparently normal segregation, with two groups of twenty two chromosomes at the poles. Five cells showed some evidence of unbalanced segregation, for instance two cells had groups of 24 + 20 chromosomes and one cell had chromosomes arranged as 23 + 20 + 1, while another two cells showed the segregation of polyvalents. Of five cells seen at anaphase two, three showed normal division, with twenty two chromosomes at each of the four poles. The other two cells each showed evidence of non-disjunction at an earlier stage of division, having the constitution (23 + 23 +21 + 21).

On the whole these results would lead one to expect that the two autotetraploids would be reasonably fertile, and evidence that this is so has already been mentioned.

Meiosis in allotetraploid hybrids

The allotetraploids used in this series of investigations were all made by crossing plants which were already auto-

tetraploid. The results of analysing pollen-mother-cell divisions are presented in Appendix G.

4n (P. vulgaris x P. elatior) M261

Of twenty three cells at metaphase one in this hybrid, each one contained at least one polyvalent, with a maximum of seven seen in one cell. This at once demonstrated that the pairing of the chromosomes from the different specific genomes observed in the diploid hybrid is a relationship between true homologues, and not a spurious kind of association like that seen in diploid P. kewensis. The similarities between the chromosomes of the two species are sufficiently great to allow them to associate with one another despite the presence of homologues from their own species. Up to seven polyvalents have been seen in the same cell, a situation to be compared with the autotetraploids, where the maximum number is eight. The number of polyvalents in the allotetraploid is therefore a high one. Plates 14 and 15 show some of the types of polyvalents seen in this hybrid.

Although they are difficult to analyse, it seems probable that some of the associations in this hybrid contain more than four chromosomes. Cells with chains of five and six chromosomes have been seen. This is not altogether unexpected in view of the formation of polyvalents

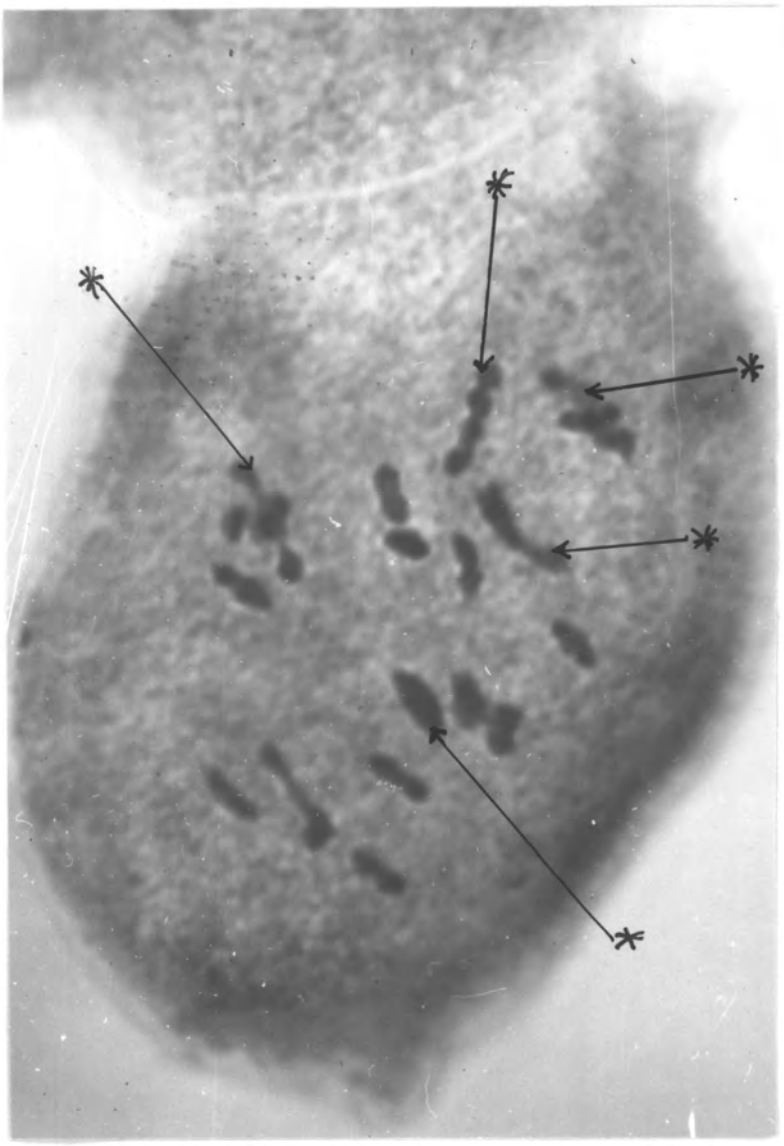


Plate 14. 4n (P. veris x P. vulgaris).

Five polyvalents at M1.

x1250

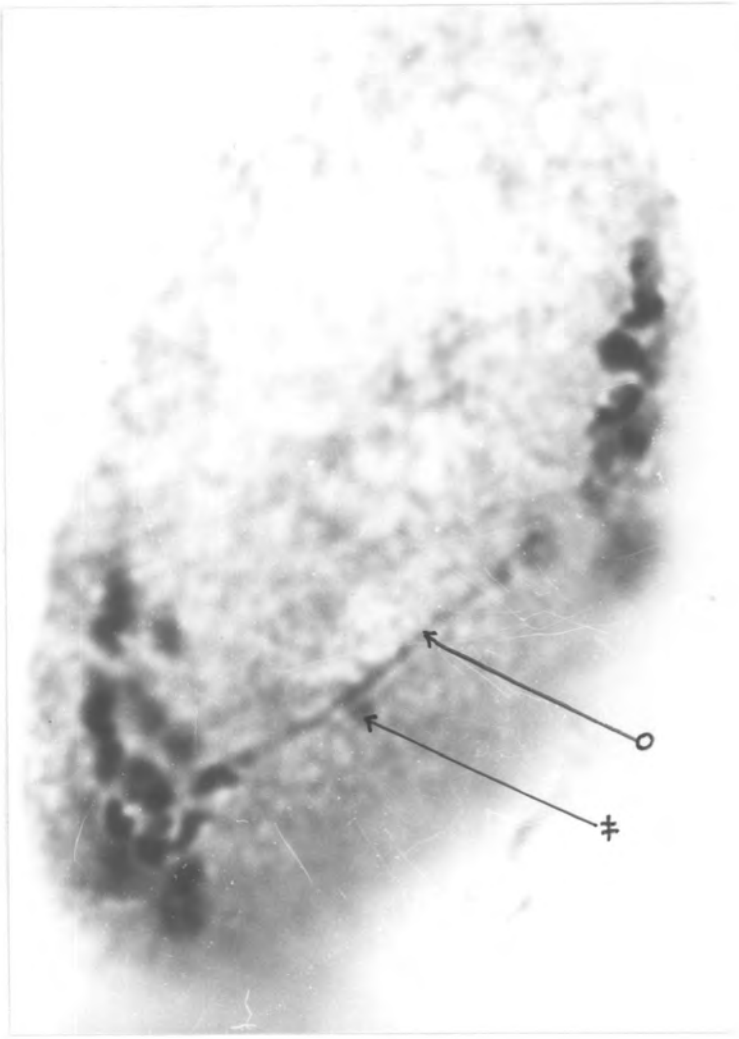


Plate 15. 4n (P. veris x P. vulgaris).
Anaphase I with inversion bridge° and fragment‡.

× 1250

in the diploid hybrid. Theoretically, in view of the known presence of translocations, it would be possible to obtain up to eight chromosomes associated in this way.

The mean number of polyvalents per metaphase cell is 2.9.

Only three cells have been seen at a post-metaphase stage, and two of these were abnormal. One cell, with twenty two chromosomes at each pole, had two chromosomes connected by a non-disjunction bridge, while the other cell had twenty one chromosomes at each pole, and an undivided bivalent on the equator. These abnormalities undoubtedly occur because they involve pairing between chromosomes which differ from one another by translocations, and therefore must be from different specific genomes.

The upset to pollen formation occasioned by abnormalities of division produced by the pairing of chromosomes from the different specific genomes and their subsequent non-disjunction, or wrongful separation, may be seen in the examination of the tetrads. In another allotetraploid (P. vulgaris x P. elatior), 28% of the tetrads were observed to be abnormal when examined after being stained with acetocarmine. The types and numbers of abnormalities are presented in Table 29, while some of the abnormalities are illustrated on plate 12.

Table 29

Numbers and types of abnormalities in tetrads of 4n (*P. vulgaris* x *P. elatior*) (M255)

Normal	Abnormal			
	4 + 1	4 + 2	3 + 1	3 + 3
80	22	6	2	1

Total abnormal = 31

Percent normal = 72.1

Meiosis in allotetraploid (*P. vulgaris* x *P. veris*)

A similar picture to that in 4n (*P. vulgaris* x *P. elatior*) is presented in the data for meiosis in this hybrid. Any difference is a difference of degree rather than of kind. Of forty six cells analysed during metaphase one, sixteen had twenty two bivalents, while twenty nine showed some signs of polyvalents. This hybrid again shows that the pairing of the chromosomes seen at metaphase one in the diploid hybrid is a pairing of true homologues, and so differs from the *P. kewensis* model. The difference in this case from that of 4n (*P. vulgaris* x *P. elatior*) is that the relationships of the genomes would appear to be a more distant one, with a good number of cells presumably showing pairing only within the specific genomes, and so giving twenty two bivalents.

Again, in this allotetraploid, as in 4n (P. vulgaris x P. elatior), the maximum number of polyvalents in one cell was seven. Another similarity between the two hybrids is the appearance of some configurations which apparently contain more than four chromosomes. Thus, seven cells have been observed to contain associations of either five or six chromosomes. This indicates that the translocations which were observed in the hybrids at the diploid level are again playing their part in the tetraploid. The mean number of polyvalents per cell is 1.8.

Plate 14 shows a cell in this hybrid with five polyvalents.

Thirty five cells were observed at post-metaphase stages. Of thirty four cells at late anaphase one, sixteen showed regular disjunction, with twenty two chromosomes proceeding to each pole. Evidence of non-disjunction is found in five cells which show groups of twenty three and twenty one chromosomes at the poles, and in three cells showing groups of twenty four and twenty. It is difficult to explain the cell containing twenty two and twenty, with two other chromosomes separated from the main body. However, this type of phenomenon, which might be due to interference with the operation of the spindle, for example, is seen in other cells with twenty two plus seventeen and five laggards,

and twenty two plus twenty one with one laggard, seen in four cells.

Other evidence of the pairing of chromosomes from the different genomes seen at anaphase one is the cell with 23 + 21 and a non-disjunction bridge. Presumably the imbalance of numbers is due to the pairing of not completely homologous chromosomes and their movement to the same pole, instead of disjoining.

The single cell containing an inversion bridge and fragment (Plate 15) is added evidence of the pairing of chromosomes from the different specific genomes, for this result of crossing over in the loop formed by the pairing of homologous parts of chromosomes differing from one another by an inversion was found in the diploid hybrids where chromosomes from the different parental genomes were paired.

One cell has been seen at anaphase two, with four groups of twenty two chromosomes.

The types of upset to tetrad formation which result from abnormalities of division are represented in Plate 32.

In Table 30 are presented the data for pollen fertility in this allotetraploid as judged by the acetocarmine staining method.

These data demonstrate that apparently fertile pollen

may be produced from this allotetraploid in quite high proportions.

Table 30

Pollen fertility in 4n (P. vulgaris x P. elatior)

Plant	Normal cells	Abnormal cells	Total	% normal
A6a	115	23	138	83.4
A6b	427	73	500	85.4
D232 (10 plants)	2925	428	3420	85.5

Meiosis in allotriploid (2n P. veris x 4n P. elatior)

This hybrid, like the others was available from Valentine's experiments into the nature of seed incompatibility.

Allotriploid hybrids were used by Peto (1934) and Stephens (1942) to determine the degree of homology of the specific genomes they were investigating. In the absence of information on the behaviour of other triploids in the present investigations, broad indications only of relationships are all that may be deduced from this data.

The number of types of configuration to be produced is likely to be variable, again depending upon chromosome

length and chiasma frequency. Darlington (1937) quoted in Table 26 has represented some of the variability to be expected in autotriploids, and he has also (ibid), summarised some of the evidence for the type of pairing to be expected in some triploid hybrids. His summary is presented in Table 31.

Also quoted by Darlington is the evidence of Kihara and Nishiyama (1930), on pairing in the allotriploid produced by crossing Triticum aegilopoides (n = 7), and T. dicoccum (n = 14). Variation of pairing during meiosis means that from 0 to 3 trivalents, from 4 to 7 bivalents, and 6 to 7 univalents have been observed.

Stephens (1942) used pairing in triploid plants produced by crossing three different diploid plants with a single tetraploid to determine the relationships of the diploid genomes. The results of his investigations are given in Table 32.

From these results Stephens was able to conclude that G. sturtii is much more closely related to G. arboreum than is G. raimondii or G. amourianum, but that the relationship is nevertheless not of a very high order.

Peto (1934) has reported on meiosis in two triploid plants which are hybrids between Festuca and Lolium, and also on the hybrid between them. The percentages of

Table 31

(from Darlington, 1937)

Pairing in triploid hybrids

AAB types (crosses of diploids with tetraploids or unreduced diploids

Authority	Genus	Basic number	Species crossed	Hybrid	Pairing ca.
1. Hakansson (1929)	Salix	19	viminalis (19) x caprea (19)	(vc x 2)	16-19 III
2. Muntzing (1930)	Galeopsis	8	pubescens (8) x speciosa (8)	(pss)	18II + 8I
3. Hollingshead (1930)	Crepis	-	capillaris (3) x tectorum (4)	(cct)	3II + 4I rarely 11II
4. Karpechenko (1927a & b)	Raphanus x Brassica	9	R. sativus (9) x B. oleracea (9)	(rrb)	(9II + 9I)
5. Steere (1932)	Petunia	7	hybrida (14) x axialaris (7)	(hha)	7III

chromosomes in different configurations found by Peto are given in Table 33.

Table 32

Data of the number of trivalents in hybrids between diploid Gossypium and the artificial autotetraploid of G. arboreum (= N14)

(from Stephens, 1942)

	Number of polyvalents per P.M.C.				
	0	1	2	3	4
N14 x G. raimondii	22	0	1	1	1
N14 x G. sturtii	10	5	3	2	0
N14 x G. amourianum	15	0	0	0	0

Table 33

Percentages of chromosomes in various configurations in triploid hybrids of Lolium and Festuca (from Peto, 1934)

Plant number	Chromosome number	Metaphase configurations		
		Univalents	Bivalents	Trivalents
Ba-174	21	38.1%	41.3%	20.6%
Bx-54	21	25.6%	51.1%	23.3%
58-bE-1	20	8.4%	28.5%	63.0%

The results are from the analysis of six metaphase nuclei in each plant

Data from the triploid (2n P. veris x 4n P. elatior)

The results of scoring the pollen-mother-cells of this hybrid are presented in the Appendix H. From this it will be seen that there are trivalents, bivalents and univalents formed, as might be expected, as well as one or two higher associations of chromosomes. The numbers and types of these configurations are recorded in Table 34 and illustrated in the Figs. 9 and 10.

Table 34

Percentages of chromosomes in various configurations in triploid hybrids of P. veris and P. elatior

Plant number	Metaphase configurations VI	IV	III	II	I	Total	
F141 (24 cells)	-	8	333	328	123	792	No. chrom.
		1.0	42.0	41.4	15.5	99.9	Percentage.
G405 (19 cells)		8	243	240	136	627	No. chrom.
		1.27	38.75	38.2	21.6	99.9	Percentage.
F151 (7 cells)	6	-	96	92	37	231	No. chrom.
	2.59	-	41.55	39.82	16.01	99.9	Percentage.
Total for the hybrids	0.36%	0.96%	40.7%	40.0%	17.9%	99.9%	

One thing which is obvious from the bulked data, and which may also be seen in some of the records for individual

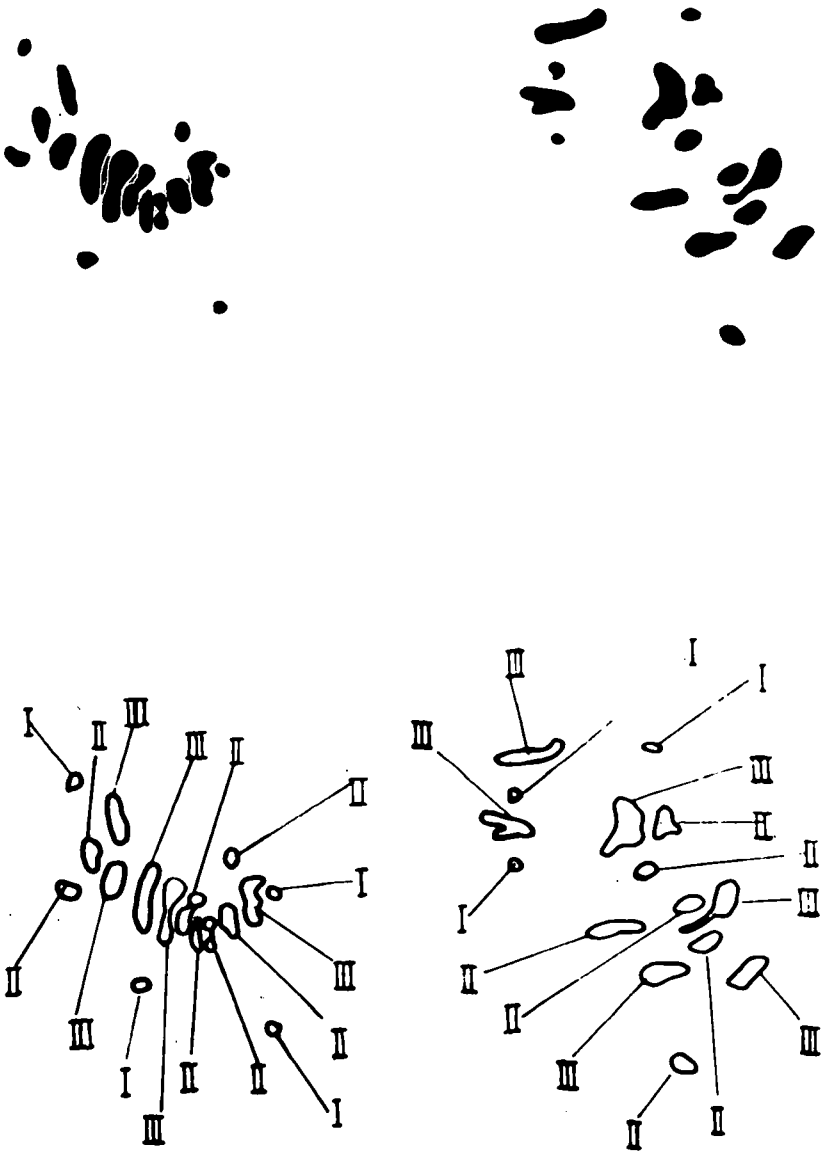


Figure 9. Meiosis in allotriploid, (2n P.veris x 4n P.elatior) Camera lucida drawings of metaphase 1.

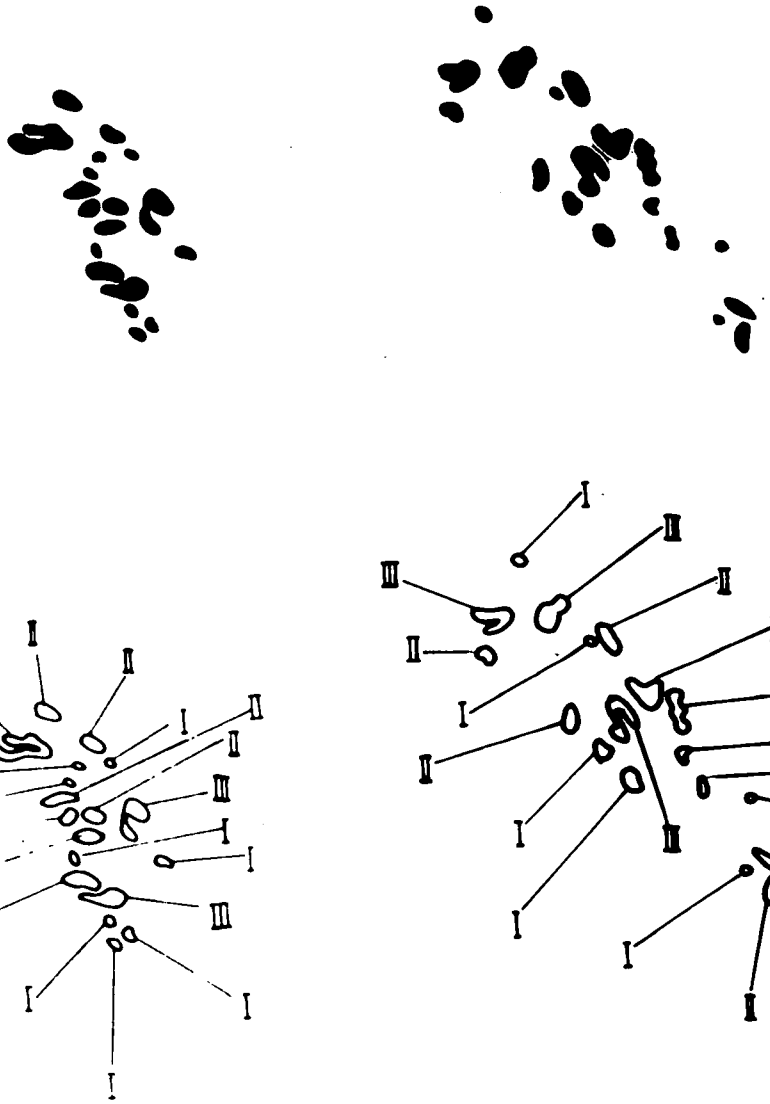


Figure 10. Meiosis in allotriploid, (2n P.veris x 4n P.elatior)

Camera lucida drawings of metaphase 1.

In the case of chromosome sets 7-11, pairing of homologues is complete, so that five trivalents are formed. Chromosome sets 3-6 do not show complete pairing so that four bivalents and four univalents result.

The nub of the argument concerns chromosome sets 1 and 2. The two parental genomes forming the sets, one diploid and the other haploid, differ from one another by reciprocal translocations. As a result of such translocations it would be possible to produce in theory a configuration containing all six chromosomes, but this is unlikely as it would require at least five chiasmata. The association of chromosomes in pairs, requiring only three chiasmata, is a much more likely event in this hybrid. The hypothesis accounts for the observations in the triploid cell, and is supported by observations at meiosis in the diploid hybrid, when translocations have been observed between these two specific genomes.

Another of the features of chromosome association in this hybrid may also be accounted for in a similar way. One or two cells have been observed to have more than three chromosomes associated together, and this too can be explained by the maximum association of homologues where translocations are again involved.

The commonest type of trivalent observed is that

produced by a linear arrangement of the chromosomes. This could only arise due to the terminalisation of chiasmata, which must indicate a fair degree of homology between the chromosomes involved.

The majority of the cells investigated (66.6%), contained polyvalents of some kind, with a maximum of eight in one cell. An average cell contained five trivalents, six bivalents and six univalents. This presumably indicates a fairly high degree of similarity between the two specific genomes.

The results of examining the tetrads of the allotriploid are presented in Table 35.

Table 35

Numbers and types of abnormality in tetrads of R242 (P. veris x 4n P. elatior)

Normal	Abnormal						
	4 + 1	1 + 1	2 + 1	2 + 2	3 + 1	4 + 2	3
87	13	1	3	2	1	1	1

Total cells 109 : Percentage normal 79.8

A great deal of caution must be exercised when interpreting the data on tetrad abnormality presented in Table 35. Although a large number of tetrads appear to be normal when stained with acetocarmine, it is not possible to be sure how

many of them contain a normal haploid number of chromosomes, and indeed it is certain that all of them cannot do so. It is really only possible to classify them as abnormal when one or two chromosomes have been left out of the major groups, and give for example 4 + 1. Presumably the small extra nuclei are due to chromosomes unpaired at metaphase one which have been distributed at random during the first anaphase, with some loss due to lagging (Mather, 1935).

DISCUSSION

The different approaches to the problem of producing a classification of this group of Primulas have produced different answers. The formal approaches have given several different arrangements of the species, and the various biosystematic investigations have similarly given different views of the taxa. The latter differ not only from the formal view, but also among themselves. Has any of these approaches, formal or experimental, any validity, and if so, which? By validity of classification one is thinking in biological terms of a natural classification which indicates the relationships of the species to one another.

The cytological evidence of meiosis in the diploid hybrids suggests that P. vulgaris has as its closest relative P. juliae, with 89% of cells at first metaphase being apparently normal. The next closest relative of P. vulgaris is elatior with 75% of metaphase cells normal, and the latter is closer to juliae with 96% of the metaphase cells normal in their F1 hybrid. The picture revealed is of a group of three fairly closely related species, P. vulgaris, P. elatior and P. juliae, only somewhat distantly related to P. veris.

It is difficult to position the other species accurately in the absence of several hybrids, and also due to their closeness to one another. The impression is, however, that

they are indeed closely related to one another, and also to P. elatior.

The tetraploid hybrids, (P. vulgaris x P. elatior) and (P. veris x P. vulgaris), show similar sorts of abnormalities during meiosis. However, 4n (P. vulgaris x P. elatior) shows a higher average frequency of polyvalents per cell (2.9), than 4n (P. veris x P. vulgaris), (1.8). From this one deduces that P. vulgaris is more closely related to P. elatior than it is to P. veris, since one would expect a higher frequency of polyvalents where the chromosomes complements have more in common with one another.

It is apparent from this evidence that the polyploids indicate the same broad sequence of relationships of the species as does the diploid data. Namely that the species P. vulgaris, and P. veris, differ more from one another than either does from P. elatior. Both of the allotetraploids as well as the allotriploid, show evidence of pairing between chromosomes of the different specific genomes which they contain, despite the fact that in the allotetraploids each chromosome has a homologue from its own specific genome available for pairing. Evidence of interspecific pairing is afforded by the presence of polyvalents in all of the hybrids studied. This is supported in the allo-tetraploid (P. veris x P. vulgaris) by the observation of an

inversion bridge and fragment in one cell, since this is the type of interspecific pairing seen in the diploid hybrid. It is obvious therefore, from all of the polyploid hybrids, that a great deal of "overpairing", or the association of chromosomes from different parental genomes has taken place. Stebbins (1947), classifies polyploids which contain two specific genomes possessing in common a considerable number of segments or even whole chromosomes, but differing from one another by a sufficiently large number of genes or chromosomal segments to produce sterility in the diploid level, as segmental allopolyploids. According to Stebbins, such segmental allopolyploids are formed only between closely related genomes. Each of the allopolyploids reported here can be placed in category of segmental allopolyploids. The formation of polyvalents shows that the genomes composing each allopolyploid have in common a considerable number of segments, or even whole chromosomes. This is further confirmation of the unity of the group, for it would appear that even P. vulgaris and P. veris, which on both the diploid and polyploid pairing evidence are the most distantly related pair of species, are nevertheless very closely related to one another.

The genetic analysis is not of very much value in ascribing relationships to the species. If the work on the

variations of dominance of stickiness of seed could be substantiated, then this might be of value in establishing the comparative closeness of P. vulgaris and P. juliae. Other features such as the presence/absence of peduncle are not in themselves sufficient to warrent the establishment of relationships. The clearest picture of relationships depending upon genetic phenomona is that given by the incompatibility data, see Table 2. Ignoring P. veris, then there is a great deal of similarity between the relationships expressed by the strength of the seed incompatibility mechanism and the cytological data. The difficulty arises in trying to fit P. veris into the picture, for the seed incompatibility mechanism gives its nearest relative as P. vulgaris, while the evidence from cytology is that P. vulgaris is the most distant relative. The cytological evidence is that the relationships of the other species with P. veris is the complete reverse of the relationships expressed by the strength of the seed incompatibility mechanism. However, the exercise using morphological data to produce a dendrogram of relationships, and based on numerical taxonomic techniques, also produced a picture of the relationships of the taxa. In this case there is no support for the order of species based on the seed incompatibility evidence. For the purposes of argument the order of the majority of the species will be ignored.

The main point is that P. vulgaris which is the most closely related species to P. veris on seed incompatibility data, is the most distantly related species to the latter on numerical taxonomic evidence.

Another morphological and anatomical analysis dealing with such species differences as the cortical parenchyma of the root, and the pith cells, is that of Fey (1929). He concluded that, "In several characters, P. elatior resembles F2 hybrids between P. veris and P. vulgaris. In other words, he found that P. elatior is in some degree intermediate between P. vulgaris and P. veris, a conclusion in accord with the present work, both the cytological evidence, and that from the numerical taxonomic analysis. Fey suggested that the reason for the intermediate position of P. elatior was that it had arisen as the result of hybridisation between the other species. Valentine (1961) has also noted the similarities of the elatior group to P. veris. He states, "On the basis of morphological characters, P. veris would appear to have much in common with P. lofthousei, which it resembles in many respects, although not in capsule characters".

It has been shown that the cytological and genetical evidence support Valentine's view that the group constitutes a single coenospecies. There are some discrepancies in

the exact order of the species, but no argument of the overall closeness of relationships within the group. This evidence is therefore somewhat at varience with the formal taxonomic viewpoint, expressed by Wendelbo (1960), and others, and described earlier. Although all of the taxa are included in the same subgenus by Wendelbo, they are split into three sections in a fashion which cannot be supported by the present investigation, nor by Valentine's seed incompatibility results. For instance, one of Wendelbo's sections in the subgenus is for P. juliae. The evidence from genetics, cytology and seed incompatibility, is that this species is much more closely related to P. vulgaris than any of the species put into the same category as the latter by Wendelbo.

Another problem concerns the taxa of the elatior group, whose rank as species or subspecies has varied. All are very closely related to one another but each can be distinguished morphologically, and occupies a distinct geographical locality. Each has developed chromosomal differences from the others, and shows the symptoms of seed incompatibility in Flhybrids with other species. There seems, therefore, to be no reason why each should not hold the rank of good species.

Of P. megaseaefolia, Smith and Fletcher (1947), say,

"Its position has been variously assessed. Balfour regarded it as an isolated member of the genus, and proposed for it a separate section. In this he was followed by Smith and Forrest (1928), who suggested however, that it tended towards P. juliae and therefore towards section Vernales. This opinion was fully confirmed by the cytological analysis of Bruun. The work of Bruun, as well as the geographical distribution favours its inclusion within the Vernales not far away from P. juliae." Unfortunately, hybrids of P. megaseaefolia are available only with two of the other species, P. elatior and P. pallasii, although Smith and Fletcher (1947) include the latter as a sub-species of P. elatior.

Observations on the hybrid (P. pallasii x P. megaseaefolia) F1, during meiosis show no signs of abnormalities during metaphase, pairing being apparently quite normal, however the presence of non-disjunction bridges at anaphase one reveals that the two genomes differ from one another by at least one translocation. With P. elatior, P. megaseaefolia again shows signs of one translocation, this time by the appearance of a quadrivalent at metaphase. However, only four percent of the metaphase cells show signs of this abnormality, so that on this evidence the taxa can be taken to be indeed closely related to one another. Although it is not possible to ascribe exact positions to the majority

of the species, in the absence of many hybrids, it is of interest to note that P.juliae and P. megaseaefolia show approximately the same percentage of abnormalities in crosses with P. elatior, 95.8 and 96.0 percent respectively.

Primula lofthousei would also appear to be a good member of this section, the cytological information on metaphase cells show eleven bivalents in crosses with P. amoena, while in a cross with P. elatior 57% of metaphase cells are normal. No other hybrids involving this species are available, but the numerical taxonomic analysis shows that phenotypically it is very closely related to the elatior group, its closest relative being P. veris.

The difficulties of classifying the taxa outlined above all spring from the use of different methods of approach to the problem. Different approaches to the classifications of organisms are adopted by different investigators, each of whom has his own concept of what constitutes a species. Similar problems have been found in a number of groups. Clausen and Heisey (1958), illustrate their discussion of this kind of problem by referring to Silene cucubalis and S. maritima, and also to Geum urbanum and G. rivale.

S. Maritima has green herbaceous bracts, erect and single flowers, many non-floriferous sterile shoots and a well developed para-corolla,inside the throat of the flower.

It has decumbent-prostrate stems, and is late blooming. S. cucubalis, on the other hand, has white coriaceous bracts, free flowering stems with well developed inflorescences, nodding flowers and only a rudimentary paracorolla. It has ascending-erect stems, and is relatively early blooming. The two taxa are in other words morphologically quite distinct. The F1 hybrids between them are, however, completely fertile, and no difficulty is experienced in producing succeeding generations.

Some taxonomists have fixed their attention on these distinct morphological differences, and have named the two as distinct species. Others, recognising their close relationships, have considered them to be subspecies, or varieties of one species. There is in other words, difficulty in reconciling evidence obtained from an experimental approach with that of the formal taxonomist.

The Geum situation is similar, Clausen and Heisey note, since the common avens and the water avens have not yet acquired full species-hood. Although the two are distinct morphologically, and have different flowering-times, they are wholly interfertile, so that they are really only morphologically distinct ecological subspecies.

Actually the Geum situation is even more like the Primula situation than Clausen and Heisey state. Far from

being "wholly interfertile", the two species show differences in fertility between reciprocal interspecific crosses. Marsden-Jones (1930) states that he tried for two years to make the hybrid with G. rivale as the seed parent, "but failed to obtain a single seed". Valentine (1951) has confirmed that the cross is successful only when G. urbanum is the female parent.

The morphological characters which distinguish these taxa of Geum are of the kind which are of primary importance in other plant families, distinguishing even different genera from one another. In Geum, as in the other groups, the characters are controlled by systems of multiple alleles.

"In a biosystematic sense these morphological characters serve as markers of ecologically separated subspecies, rather than as markers of distinct species. Some taxonomists may simply refer to them as species however", (Clausen and Heisey, 1958).

This is relevant to the Primula situation, for it serves as a reminder that taxa evolve, and are evolving, and do not necessarily show the distinctness from one another which may be looked for by the formal taxonomist. Alternatively, they may have the morphological appearance of distinctness without any of the barriers to prevent the formation of perfectly fertile hybrids when they meet. The

examples of Silene and Geum show that there may be difficulties in reconciling the evidence from different methods of establishing status for taxa. One approach lays stress on the formal observation of selected morphological characters, together with ecological and geographical distinctions. The other takes into consideration other data which has a "biological" significance. Davis and Heywood (1963), summarise the situation as follows:- "Several classifications of species definitions have been proposed, cf. Mayr (1957); Baudry (1960) - so involved has the subject become, but the two main ones are:-

(a) Taxonomic (embracing the orthodox, typological, morphological, morphogeographical, etc.).

(b) Biological (including the biosystematic, genetical, cytogenetical, non-dimensional, multi-dimensional, etc.)".

This classification is relevant to the present work, for it describes clearly the different methods of approach to the problems of classification. Valentine's approach of investigating the seed incompatibility relationships of the taxa, like the present investigation of genetical and cytological relationships, has led to a biological picture, which although not itself unambiguous, clearly differs from the classical morphological picture.

Since they use different methods of analysis, and since

in any case their aims are different, one might expect that the different approaches, taxonomic and biological, would yield different results, similar to those seen concerning taxonomic status seen in Silene and Geum.

Part of the difficulty of expressing relationships is that any attempt is almost bound to be more simplified than the complex situation reviewed, especially if the taxa have but recently diverged, and are still in the process of divergence. Different rates of evolution will lead to the very rapid morphological and ecological divergence of the two taxa, while yet another pair may remain very similar in the absence of any strong pressures. All this while other mechanisms concerned with the isolation of the species are diverging at other rates, or not at all. Hence different ways of expressing the relationships of species may reflect differences which are simply due to different rates of evolution, and have very little to do with degrees of relationship as such.

The reliance placed by some authorities on the extent of chromosome pairing in interspecific hybrids as an index of relationships of the species whose genomes are concerned has already been mentioned. Many of these workers refer to Stebbins (1938) and his work on Paeonia as justification for this approach. Paeonia is a very bad example to choose

for this purpose, however, for Stebbins notes that the pairing affinities of the chromosomes is of little or no value for determining the phylogenetic relationships between the species of this genus. The morphology of the chromosomes does not help, nor the degree of structural differentiation between the chromosomes of different species, as deduced by the amount and type of abnormality at meiosis in hybrids. Although there appears to be some correlation between structural hybridity and failure of pairing, this does not help, for one or other may be present alone, not only in hybrids, but in species.

Stebbins notes that such chromosomal structural changes as inversions have not been effective in differentiating species in the absence of other agents. However, they have resulted in discontinuity, which has been responsible for the formation of varieties within the species.

The matter is complicated in Paeonia by the number of abnormalities seen in the pure species. Since each species is apparently a heterogeneous collection of different karyotypes, it is difficult to use the differences between two specific karyotypes as evidence of their degree of relationship, for it cannot be clear that one is dealing with representative karyotypes. In Primula on the other hand, there is no reason to believe that the karyotypes of

the different taxa are not representative. The absence of abnormalities in the pure species is support for this view. Nevertheless, the differences between specific genomes may be as much a measure of the rate of evolution as a measure of the time of divergence of the species involved. Greater abnormalities could occur between the genomes of species of comparatively close relationship in time, which had diverged rapidly under the influence of strong selection pressure, than between species which are not as closely related in time, but which had not been subjected to such strong selection pressures. Chromosome studies do not necessarily show anything more about the evolutionary relationships of taxa than the speed of their divergence, and hence do not necessarily show the natural order of the taxa considered.

Similar arguments can be advanced against the claims of any particular method which is said to illuminate the relationships of the species, and reveal the evolutionary truth. In all cases the degree of relationship revealed may simply reflect the strength of the pressures which gave rise to the features under consideration. This in turn may reflect the difference between allopatric and synpatric speciation. The former would not require such strong barriers as the latter to prevent hybrids forming, and hence

speciation could proceed in the absence of internal barriers, or in the presence of only slightly developed ones.

The seed incompatibility mechanism itself has not necessarily arisen by direct selection pressure for it. Indeed it is difficult to imagine how there could be direct selection for the mechanism. The phenomenon is a post fertilisation one and as such the maternal plant which manifests it will be less fertile than one which does not, when producing hybrid seed. Hence the reduced production of seed in such crosses would mean that could be no selection for the mechanism in the species, since its success would mean the reduced fertility of the individuals developing it.

Woodell (1960) has shown that "minor differences in timing and seed size occur" in the development of the embryos of P. vulgaris, P. elatior and P. veris. These differences are not necessarily the results of direct selection, but may be merely developmental consequences of differences in ecological requirements. For instance, Valentine (1948) quotes Hegi (1931), who regards the primrose as a thermophilous species, which requires a relatively mild winter and spring, while the oxlip can withstand low winter temperatures. Consequently, the primrose is more Western, and the oxlip more Eastern in distribution. As a result

of the differences, the species have apparently come to differ "for different rates and temperature optima for various processes of cellular metabolism", which Stebbins (1966) says may give rise to "genic disharmony in the development of hybrids". In the F1 hybrid between two species, the relevant tissues are the endosperm and the embryo. The endosperm is triploid, and contains two representatives of each chromosome of the mother, and one from the male parent. The endosperm will therefore be different in gene content in reciprocal crosses between the same two parents. Woodell and Valentine (1963) write, "Seed incompatibility, which is the partial or complete failure of seed development after fertilisation has taken place, is the result of an interaction between genetically different paternal and maternal tissues; the physiological incompatibility and its morphological expression in the abnormal seed must have a physiological expression". The same authors (1963) write, "The growing embryo thus needs a series of growth factors which are probably produced in the endosperm, and if these fail the embryo will stop growing and may die. Again it is possible that the nature and production of these growth factors are different in different Primula species, so that in an interspecific cross the embryo fails to grow or develops abnormally".

Seed incompatibility could therefore be due to different rates of development of endosperm and embryo,

due to the interaction of the genes of the two parents which differ in their rates of growth. As a result, the lack of coordination between these two tissues could result in the development of an abnormal embryo. The results would be expected to differ in reciprocal crosses due to the balance between the parental genomes being different in the triploid embryo. Doubling the number of chromosomes of both parents might be expected to have no significant effect on development, but doubling the chromosome number of only one might. The triploid hybrid (2n *P. veris* x 4n *P. elatior*) represents the only way in which it has been practicable to cross these two species, and the success might be a consequence of the increased gene dosage of *P. elatior* effecting the rate of development of the embryo or endosperm. Be that as it may, the postulated differences in metabolic rate in the cells of the various species are not necessarily the result of competition between the species during development, which might give a clue to relationships, but merely the consequence of adaptation to different ecological conditions. Since in the latter case the differences could be secondary features of speciation, the differences between the species would not necessarily serve as good indicators of evolutionary relationships.

This argument does not preclude the possibility that

seed incompatibility does in fact indicate evolutionary relationships. However, it does present an alternative to this view, which is at least equally as likely.

CONCLUSIONS

If the aim of taxonomy is simply to enable one to identify individual taxa, then this is achieved as soon as they are described. That this is not the only aim is shown by the constant revision of their level of importance, and of their relationships with other taxa. The initial description simply starts the phase of the 'alpha' taxonomy of Turrill, which is followed by other efforts to produce more "meaningful" relationships within the groups studied. As a result, new revisions are published as new people examine the evidence available to them. As Solbrig (1966) puts it, "..... the work of the systematist does not stop with description and classification of species. He wants to know the genetic relationships and history of the species he works with and the mechanisms which brought them into being".

The new classifications are usually based upon a particular concept which it is held has given, or is capable of giving, a greater insight into the relationships of the group.

However, once several lines have been investigated, say pollen morphology, seed incompatibility, cytology, one may have several different possible "evolutionary" series to choose from. One is then faced with the problem of making a choice. Which, if any, is meaningful? The

selection of a particular line might be difficult to defend before the Monopolies Commission, to say nothing of other scientists. That a decision should be made seems reasonable, otherwise the work done will have been wasted.

It has been stated repeatedly in this work that different approaches have emphasised different features of the group in establishing categories, with the result that the categories have varied even though the material dealt with has not. It is unfortunate that this should be the case, and that there should be the repeated raking over of the ashes of an alpha taxonomy, and it is difficult to provide an answer based purely on biosystematic results, for they are also to some extent contradictory.

The greatest overlap between classical taxonomy and biosystematics is afforded by Wendelbo's classification, giving the subgenus Primula. "It would seem legitimate toconclude that the evidence from the hybridisation experiments supports Wendelbo's groupings of the sections Primula, Julia and Megaseaefolia into a single subgenus", (Valentine, 1962).

What difficulty there is in equating Wendelbo's system with the biosystematic data is due largely to his further division of the group into sections. If these are dropped, and the order of species is taken to be the

easily repeatable one of the numerical taxonomic data, where no characters are especially emphasised, then his subgenus can be taken to incorporate the following species:-

P. veris L.

P. lofthousei Harr.

P. pallasii Lehm.

P. elatior (L) Hill

P. amoena M. Bieb.

P. intricata Godr. et Gren.

P. megaseaefolia Boiss.

P. juliae Kusn.

P. vulgaris Huds.

Each species is a morphological entity, and also has the status of an ecospecies.

In this way the results of the formal taxonomic approach can be brought together with those of the experimental approach, to give a synthesis which one would hope to be at least a step on the road towards an 'omega' taxonomy for the taxa considered.

It is appropriate to end with a quotation from Hull (1967): "Every taxonomy is a provisional and implicit theory (or family of theories). As knowledge of a particular subject-matter grows, our conception of the subject-matter changes; as the concepts become more fitting,

we learn more and more. Like all existential dilemmas in science, of which this is an instance, the paradox is resolved by a process of approximation: the better our concepts, the better the theory we can formulate with them, and in turn, the better the concepts available for the next, improved theory".

APPENDIX A

Peduncle presence/absence scored at different times in H30. F2 (vul. x elat.)

Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit
1	•	-	36	±	±	71	-	-	F	+	+
2	•	•	37	±	±	72	±	±	G	-	-
3	•	•	38	-	-	73	-	•	H	+	+
4	+	+	39	•	•	74	+	+	I	-	-
5	+	+	40	±	±	75	±	-	J	-	-
6	±	±	41	±	±	76	±	±	K	±	±
7	•	•	42	-	-	77	±	±	L	+	+
8	-	-	43	±	±	78	-	-	M	±	-
9	+	+	44	±	±	79	+	•	N	+	+
10	-	-	45	±	-	80	±	∓	O	-	-
11	•	•	46	-	-	81	±	∓	P	±	-
12	+	+	47	±	•	82	±	∓	Q	±	-
13	-	-	48	±	±	83	±	-	R	+	+
14	+	+	49	±	-	84	±	∓	S	+	+
15	+	+	50	±	•	85	+	+	T	+	+
16	•	•	51	+	+	86	±	∓	U	+	+
17	+	+	52	+	+	87	+	+	V	•	•
18	+	+	53	+	+	88	±	∓	W	•	•
19	±	+	54	±	+	89	±	∓	X	-	-
20	±	-	55	+	•	90	+	+	Y	±	±
21	+	+	56	•	•	91	±	∓	Z	±	±
22	-	-	57	-	-	92	±	∓	a	±	±
23	-	•	58	±	+	93	+	+	b	±	±
24	+	+	59	•	•	94	-	-	c	±	+
25	-	-	60	+	+	95	•	•	d	-	-
26	-	-	61	-	-	96	+	+	e	±	+
27	∓	+	62	±	±	97	+	+	f	±	-
28	±	±	63	+	+	98	-	-	g	•	•
29	•	•	64	-	-	99	±	±	h	-	-
30	+	+	65	-	-	100	+	+	i	-	-
31	±	±	66	±	-	A	±	∓	j	±	±
32	-	-	67	±	±	B	-	-	k	±	-
33	±	+	68	±	±	C	-	-	l	•	•
34	+	+	69	-	-	D	+	+	m	±	+
35	•	•	70	±	±	E	±	∓	n	±	±
									o	±	±
									p	-	-
									q	±	+

APPENDIX A (contd.)

Peduncle presence/absence scored at different times in H36. F2 (vul. x elat.)

Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit
1	±	+	±	33	+	+	+	65	±	-	±	97		•	•
2	•	•	•	34	±	+	±	66	+	+	+	98		•	•
3	•	•	•	35	+	+	+	67	±	±	±	99	±	+	±
4	•	•	•	36	+	+	+	68	+	+	+	100	±	+	±
5	±	±	±	37	+	+	+	69	±	±	±	101	+	•	+
6	-	-	•	38	+	+	+	70	+	+	•	102	+	+	+
7	-	-	•	39	-	-	-	71	+	+	+	103	+	+	+
8	±	±	±	40	+	+	+	72	+	+	+	104	+	+	+
9	±	-	+	41	±	±	±	73	±	±	+	105	±	±	-
10	•	•	•	42	±	-	+	74	±	±	±	A	+	+	+
11	-	-	-	43	•	•	•	75	±	±	+	B	+	+	+
12	•	•	•	44	±	-	±	76	+	+	+	C	±	+	±
13	-	-	-	45	+	+	+	77	±	±	+	D	±	±	±
14	+	+	+	46	+	+	+	78	•	•	•	E	+	+	+
15	±	±	±	47	•	•	•	79	±	±	+	F	+	+	+
16	±	±	±	48	-	-	-	80	±	±	±	G	±	+	±
17	±	±	+	49	+	+	+	81	+	+	+	H	+	•	+
18	±	±	+	50	±	±	±	82	±	±	±	I	±	-	±
19	+	+	+	51	±	±	±	83	+	+	+	J	+	+	+
20	+	+	+	52	+	+	+	84	+	+	+	K	+	+	+
21	-	-	-	53	+	+	+	85	+	+	+	L	+	+	+
22	+	+	+	54	+	+	•	86	+	+	+	M	+	+	+
23	±	±	±	55	±	+	±	87	±	±	±	N	±	+	±
24	±	±	+	56	+	+	+	88	±	+	-	O	+	+	+
25	+	+	+	57	+	+	+	89	-	-	-	P	+	+	+
26	+	+	+	58	+	+	+	90	+	+	+	Q	-	-	-
27	±	±	+	59	-	-	•	91	±	±	±	R	-	-	-
28	+	+	+	60	-	-	-	92	±	±	±	S	+	+	+
29	±	±	+	61	±	+	-	93	±	±	±				
30	±	±	±	62	+	+	•	94	+	+	+				
31	-	-	-	63	±	±	+	95	-	-	•				
32	±	+	±	64	±	±	+	96	±	+	±				

APPENDIX A (contd.)

H44.			H43.			H41.			H45-48			H45-48		
Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit	Plant	Flower	Fruit
A	..	·	1	±		A	-	-	A	-	-	12	±	+
B	-	·	2	±		B	·	·	B	-	-	13	-	±
C	·	-	3	-		C	±	±	C	-	-	14	-	±
D	-	·	4	-		D	±	±	D	-	-	15	-	·
E	·	-	5	-		E	·	·	E	±	±	16	-	-
F	-	-	6	-		F	-	-	F	-	-	17	-	-
G	-	-	7	-		G	-	-	G	-	-	18	-	-
H	+	±	8	-		H	-	-	H	-	-	19	-	·
I	-	-	9	-		I	-	-	I	-	-	20	-	-
J	-	-	10	±		J	-	-	J	+	±	21	-	-
K	-	-	11	-		K	-	±	K	-	-	22	-	-
L	-	-	12	-		L	-	-	L	-	-	23	±	-
M	±	-	13	±		M	-	-	M	-	-	24	±	±
N	-	-	14	-		N	-	-	N	-	-	25	-	±
O	±	±	15	-		O	-	-	O	-	-	26	-	-
P	·	·	16	±		P	-	-	P	-	-	27	±	±
Q	-	-	17	-		Q	-	-	Q	-	-	28	-	-
R	-	-	18	-		R	-	-	R	±	-	29	-	±
S	-	-	19	±		S	-	-	S	±	·	30	+	-
T	-	-	20	±		T	-	-	T	±	±	31	-	-
U	-	±	21	-	±	U	-	±	U	-	·	32	-	±
V	-	-	22	-	-	V	-	-	V	-	-	33	+	·
W	+	-	23	-	-	W	-	-	W	±	-	34	±	-
X	-	-	24	-	+	X	-	-	X	-	·	35	-	·
Y	±	±	25	-	-	Y	-	-	Y	-	·	36	-	-
Z	-	-	26	·	-	Z	-	-	Z	-	-	37	-	-
H49			27	-	-	1	-	-	1	-	-	38	+	-
A	±	±	28	-	-	2	±	-	2	-	-	39	±	-
B	-	±	29	-	-	3	±	±	3	±	-	40	+	-
C	-	-	30	-	-	4	-	-	4	-	±	41	-	+
D A	-	-	31	-	-	5	-	-	5	-	±	42	-	-
B	-	-	32	-	-				6	-	-	43	±	·
E	-	-	33	-	-				7	-	·	44	..	-
F	-	-	34	-	-				8	±	±	45	-	-
G	·	-	35	-	-				9	±	·	46		±
H	-	-							10	±	±	47		+
I	-	-							11	±	±	48		-
J	-	-												
K	·	-												
L	-	-												
M	-	-												
N	+	±												
O	-	±												

APPENDIX B

Variation in the number of pedunculate inflorescences and basal flowers, in F1 (vulgaris x elatior) and F1 (elatior x vulgaris)

(Valentine - personal communication)

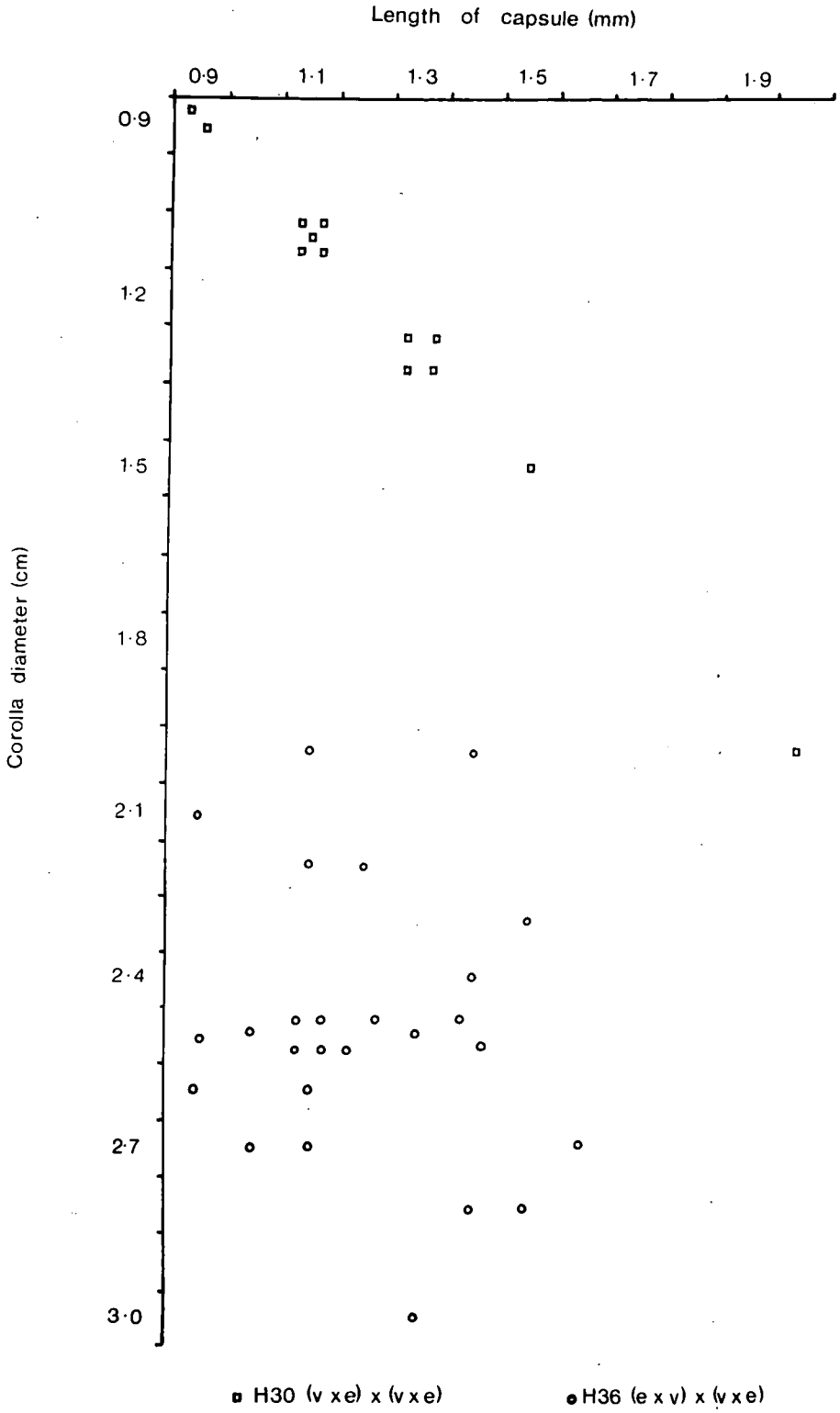
Plants were recorded over four seasons, and the two entries are not necessarily for the same season.

G89 vulgaris $585_{(3)p}$ x elatior $D13_{\gamma+}$

G90 + G91 elatior $D13_{\gamma+}$ x vulgaris $585_{(3)p}$

Code	No. of ped. inflor.	No.of basal inflor.	Code	No.of ped. inflor.	No.of basal inflor.
G89A	6	20	G90A	8	17
G89B	8	5	G90B	4	8
G89C	5	10	G90C	7	12
G89D	1	0	G90D	3	1
G89E	3	2	G90E	5	8
G89F	6	23	G90F	2	0
G89G	1	0	G90G	6	10
G89H	5	12	G90H	7	12
G89J	10	4	G91A	7	12
G89K	5	4	G91B	3	20
G89L	6	7	G91C	4	2
G89M	7	16	G91D	7	9
G89N	4	8	G91E	4	1
G89O	6	47	G91F	6	53
G89P	4	0	G91G	4	19
G89Q	4	13	G91H	4	26
Total:	81	171	Total:	81	210
Mean:	5·1 ± 2·243	10·7 ± 10·198	Mean:	5·1 ± 1·749	13·1 ± 11·767

Appendix C: Variation of capsule length with corolla diameter. F2 (vulgaris x elatior)



APPENDIX D

Chromatographic analysis of sugars associated with seeds and placenta in P. vulgaris

The secretion from the placenta was washed in alcohol, and then spotted onto Whatman's No.1 chromatographic paper. It was dried, and then run at 24°C in the solvent butanol-propionic acid-water, as used by Benson, et al. (1950) - (see below).

When the first run was complete, the paper was dried, and run in the same solvent system at right-angles to the first.

The paper was again dried, and sprayed with p-anisidine hydrochloride in n-butanol, after which it was heated to 110°C.

The position of the sugars was indicated by coloured spots.

The solvent system.

"Butanol-propionic acid-water. Fresh solvent is prepared from two solutions, A (1246 ml. of n-butanol and 84 ml. of water) and B (620 ml. of redistilled propionic acid and 790 ml. of water). A and B are adjusted to give a single phase solution which becomes cloudy on cooling to 22°C, or two degrees below the thermostated room temperature". Benson, Bassham, Calvin, Goodale, Haas and Stepka, (1950).

APPENDIX E(a)

Pairing at meiosis in Primula hybrids

	vulg. x el.	el. x ju.	ve. x el.	el. x lo.	el. x in.	meg. x el.	ve. x vulg.	ve. x ju.	ju. x in.	lo. x am.	ju. x vulg.	pa. x me.
11 bivalents, M1	59	49	34	37	49	47	63	47	22	68	62	36
11 at poles, A1 and T1	5	1	1	6	-	6	-	10	-	-	6	19
10(2) + 2(1)	24	17	48	19	5	1	17	36	5	11	36	4
9(2) + 4(1)	4	1	8	-	1	-	3	5	2	2	7	-
8(2) + 6(1)	-	-	-	-	-	-	17	1	-	-	2	-
7(2) + 8(1) and 6(2) + 10(1)	-	-	-	-	-	-	1	-	-	-	2	-
9(2) + 1(3) + 1(1)	3	-	18	7	2	-	23	4	4	6	1	-
7(2) + 2(3) + 1(1)	2	-	4	1	-	-	7	1	-	-	-	-
1 quadrivalent	9	1	2	1	2	2	32	2	3	4	-	-
2 quadrivalents	3	-	-	-	-	-	11	-	-	-	-	-
1 quadrivalent and 1 trivalent	-	-	-	-	-	-	2	-	-	-	-	-
1 quadrivalent and 2 trivalents	1	-	-	-	-	-	6	-	-	-	-	-
Bridges at A1 and T1	1	-	-	-	-	-	-	-	-	-	3	2
Bridges and fragments	1	1	-	-	-	-	-	-	-	-	1	-
Laggards at A1 and T1	2	-	-	-	1	-	-	1	-	-	5	-
10 + 12 at A1	1	1	-	-	-	-	-	-	-	-	-	-
4 11's at T2	-	-	-	-	-	-	-	1	-	-	-	-
Total cells analysed	115	71	115	71	60	56	182	108	36	91	125	61

Codes. amoena = am. intricata = in. lofthousei = lo. pallasii = pa. elatior = el. juliae = ju.
megaseaefolia = me. veris = ve. vulgaris = vul.

APPENDIX E(b)

Pollen fertility and chromosome pairing in *Primula* hybrids

	vulg. x el.	el. x ju.	ve. x el.	el. x lo.	el. x in.	meg. x el.	ve. x vulg.	ve. x ju.	ju. x in.	lo. x am.	ju. x vulg.	pa. x me.
Percentage normal pollen	75	83	43	72	75	85	32	42	79	71	60	78
Percentage cells showing 11 bivalents at M1	$^{59}/_{105}$	$^{49}/_{68}$	$^{34}/_{114}$	$^{37}/_{65}$	$^{49}/_{60}$	$^{47}/_{50}$	$^{63}/_{182}$	$^{47}/_{96}$	$^{22}/_{36}$	$^{68}/_{91}$	$^{62}/_{110}$	$^{36}/_{40}$
	56	72	30	57	82	94	35	49	61	75	56	90
Percentage cells with polyvalents at M1	$^{18}/_{105}$	$^{1}/_{68}$	$^{24}/_{114}$	$^{9}/_{65}$	$^{4}/_{59}$	$^{2}/_{50}$	$^{81}/_{182}$	$^{7}/_{96}$	$^{7}/_{36}$	$^{10}/_{91}$	$^{1}/_{110}$	$^{0}/_{40}$
	17	1	21	14	7	4	45	7	19	12	1	0

APPENDIX F.

Data of meiosis in the autotetraploids

i. 4n P.elatior

G238(6) (4n P.elatior) M1 Label 30·0/67·0

28·0/50·9	(4IV 14II)	
28·0/50·9	(8IV 6II)	(Left hand upper cell)
28·0/49·6	2 cells. (3IV 1III 13II 3I)	(Right hand cell)
	(22II)	(Left hand cell)
26·2/49·2	(2IV 2III 13II 4I)	
26·2/50·8	(1IV 1III 16II 5I)	
28·0/50·9	(4IV 14II)	
28·2/48·1	(3IV 15II 2I)	
27·1/51·9	(2IV 2III 13II 4I)	
27·0/48·1	(2IV 2III 13II 4I)	
27·0/48·2	(3IV 1III 14II 1I)	
25·6/57·5	(2IV 2III 14II 2I)	
26·0/57·6	Two cells squashed together	(Upper of two cells)
	(3IV 16II)	(Lower of two groups)
30·1/55·7	(1IV 1III 18II 1I)	
24·0/62·8	(1IV 19II 2I)	
25·8/55·1	(5IV 12II)	
26·0/50·9	(2IV 15II 6I)	
21·8/60·6		
18·1/57·9	(3IV 16II)	
16·9/53·5	(5IV 1III 14II 1I)	
16·9/55·9	(3IV 1III 14II 1I)	
16·8/56·0	(1IV 1III 17II 3I)	
16·7/55·9	(right hand of two cells (3IV 2III 11II 4I)	
17·2/64·1	(5IV 12II)	

APPENDIX F.

ii. 4n P.veris

C16b (2n = 44) AII. (Patholette 73) Started 25·9/134·1 (label to right)R.D.E.

31·9/110·6	A1 (22 + 22)
33·0/104·9	(3 cells) (4IV 2III 9II 4I) - middle cell
34·7/108·6	(right hand cell) (3IV 2III 12II 2I)
34·5/108·0	(5IV 1III 2I)
33·8/108·8	(6IV 10II)
34·1/109·4	(7IV 1III 6II 1I)
34·5/111·2	(4IV 2III 9II 4I) - upper cell
34·5/111·2	(4IV 3III 8II 3I) - lower cell
34·5/111·0	(3IV 1III 14II 1I) - left hand cell
34·5/111·0	(3IV 16II) - right hand cell
34·2/111·0	(6IV 10II)
34·5/110·9	(4IV 1III 10II 5I)
34·1/110·9	(5IV 2III 7II 4I)

C16n (1) R.D.E.

34·1/110·8	(4IV 2III 10II 2I)
34·2/110·9	(3IV 5III 7II 3I)
34·6/109·9	(6IV 2III 6II 2I)
35·1/110·1	(6IV 1III 7II 3I) (left hand cell of two)
35·1/110·1	(3IV 15II 2I) (right hand cell of two)
35·6/110·8	(2IV 1III 16II 1I)
35·6/111·6	(3IV 2III 12II 2I)
35·1/114·0	(6IV 10II)
36·1/115·0	(6IV 10II)
38·9/109·5	(3IV 1III 14II 1I)
40·1/113·0	(5IV 1III 2I) (left hand cell of three)
40·1/113·0	(5IV 1III 9II 3I) (middle cell of three)
40·1/113·0	(3IV 2III 11II 4I) (right hand cell of three)
40·5/116·0	(22 + 22 + 22 + 22)

APPENDIX F.

ii. 4n P.veris

C16n (1) D.H.V. (M2) Patholette 73.

35·0/116·0	(21 + 23)
35·1/116·8	(24 + 20)
35·0/115·6	(22 + 22)
34·9/115·9	(22 + 22 + 22 + 22)

C16n (1) R.D.E. (M1) (Started 23.0/100·6)

27·8/136·7	(6IV 2III 6II 2I)
27·8/136·8	(8IV 6II)

C16n (2)

18·5/104·8	(23 + 23 + 21 + 21)
14·1/108·0	(21 + 21 + 22 + 24)
12·5/116·8	(23 + 20 + 1)
12·1/107·6	(20 + 20 + 48)
8·1/105·1	(22 + 22 + 22 + 22)
71·8/24·1	(6IV + 10II)
72·0/23·9	(22II)
72·4/23·9	(22 + 22)
74·0/24·0	(1IV + 20II)
75·4/24·0	(1IV + 20II)
75·4/24·0	(3IV + 16II)
75·6/24·1	(3IV + 1III + 14II + 1I)
74·1/23·3	(22 + 22)
74·3/23·1	(22 + 22)
73·1/23·4	(20 + 24)
73·1/23·4	(22II)
73·0/23·3	(22 + 22)
	(22 + 22)
	(5IV + 12II)
	(8IV + 6II)

APPENDIX F.

ii. 4n P.veris

C16n (2) (contd.)

72•9/23•2	(22 + 22)
72•1/23•4	(5IV + 12II)
71•9/23•1	(22 + 22)
71•9/23•9	(24 + 20)
70•9/23•0	(5IV + 12II)
73•0/23•0	(1IV + 20II)
73•0/23•1	(4IV + 14II)
75•6/24•1	(2IV + 2III + 13II + 5I)
73•0/23•1	(7IV + 7II + 2I)
72•1/23•4	(6IV + 9II + 2I)
70•9/23•0	(5IV + 12II)

APPENDIX G.

Data of meiosis in (a) 4n (veris x vulgaris)

D232b. Slide 1 4n (P.veris x P.vulgaris) Zeiss Microscope: Label to right.

12·1/109·5	A1(22 + 22); A1(22 + 22)
9·2/110·0	(22II)
14·1/110·9	(22II)
14·1/105·2	A1(22 + 22), (22 + 22)
14·1/105·2	(24 + 20), (23 + 21)
15·1/111·1	(1IV 2II)
14·8/106·9	(22II), (22II)
10·1/103·0	(22II)
10·5/103·9	(22II)
10·9/104·0	(2IV 18II)
13·0/105·1	(22 + 22)
13·9/105·1	(22 + 22)
13·8/105·8	(1IV 18II 4I)
13·5/105·8	(22II)
14·4/105·8	(21 + 21, + 2 laggards)
14·4/105·8	(24 + 20)
14·4/105·8	(22 + 22)
14·5/105·5	(22 + 22)
14·5/105·5	(22 + 22)
14·5/105·5	A1(22 + 20, + 2 laggards)
14·7/106·0	A1(23 + 21)
15·0/106·0	(22 + 21, + 1 laggard)
15·0/106·0	(22 + 17, + 5 laggards)
14·0/105·8	A1(21 + 22, + 1) (21 + 23)
14·1/105·9	A1(23 + 21, Bridge + frag.)
14·1/105·8	(22 + 22)
14·1/115·9	(21 + 23)
13·5/105·8	(22II), (22 + 22, Bridge no frag.)
14·2/105·8,	(21 + 21, + 2 laggards) (24 + 20) (22 + 21, + 1 laggard)
14·5/105·8	(22 + 22) (23 + 21)
14·6/105·7	(22 + 22)
19·1/106·2	(22II)
9·1/110·5	(22II)
16·0/106·5	(1IV 1III 2I)
16·0/106·6	(22II)
19·2/107·1	(22II)
16·0/106·6	(22II)
14·8/117·0	(22II)
10·8/103·8	(22II)
7·0/108·0	(1IV 20II)
12·0/109·2	(22 + 22) (22 + 22)
12·0/109·8	(22 + 22)
13·0/105·0	(22 + 21 + 1 laggard)

APPENDIX G.

D232b. 4n (P.veris x P.vulgaris)

Anaphase 1 data:

22 + 22 - 16 cells

23 + 21 - 5 cells

24 + 20 - 3 cells

22 + 20 + 2 laggards - 1 cell

21 + 21 + undivided bivalent - 2 cells

22 + 17 + 5 laggards - 1 cell

21 + 22 + 1 laggard - 4 cells

23 + 21 with bridge and fragment - 1 cell

22 + 22 with non-disjunction bridge - 1 cell

Telophase 2) Anaphase 2) data:

One cell with groups of chromosomes 22 + 22 + 22 + 22

D238b. (3) Patholette 71. (label to right) 4n (P.veris x P.vulgaris)

33·9/138·3	(1IV 2III 11II 12I)
33·8/138·1	(1IV 2III 15II 4I)
34·5/139·0	(22 + 21 + 1 laggard)
34·4/139·5	(23 + 20 + 1 laggard)
34·8/102·9	(1VI 5IV 6II 6I)
34·8/102·5	(2IV 1III 11II 11I)
34·9/102·5	(1VI 1IV 15II 4I)
34·6/103·0	(22II)
35·1/103·0	(1IV 1III 18II 1I)
35·1/102·9	(3IV 13II 6I)
35·2/103·1	(1IV 2III 14II 6I)
35·5/103·1	(1IV 1III 18II 1I)
35·2/103·0	(1IV 2III 15II 4I)
36·0/103·1	(1IV 20II)

APPENDIX G.

D238b.(contd.)

39·0/137·0 (2IV 2III 13II 4I)
40·0/110·0 (1VI 1IV 12II 10I)
40·5/107·9 (22II)
39·9/109·1 (3IV 15II 2I)
41·1/104·2 (5IV 10II 4I)

D232 (7b) (Started 22·4/124·0) 23·1/112·9 (1VI 2IV 2III 1III 2I)

D232. (Slide A) 40·8/107·9 (1IV 3III 12II 7I)
40·4/113·2 (1VI 4IV 2III 6II 4I)
41·6/112·5 (1VI 3III 13II 3I)
42·2/110·1 (1V 1IV 4III 1III 1I)
42·9/108·0 (2IV 2III 14II 2I)

D238. (Slide 10. 43·1/102·1 (5IV 1III 10II 1I)
42·9/114·9 (2IV 3III 12II 3I)

Jan.65. Patholette 71. A6b (1) 4n (P.vulgaris x P.veris)
33·4/127·4 (1IV 3III 13II 5I)

APPENDIX G.

Data of meiosis in (b) 4n (vulgaris x elatior)

M261. (Slide 2) Patholette 71. (4n P x O)

41·0/-101·6	(2IV 18II)
40·8/-101·1	(1IV 2III 16II 1I)
39·0/-104·8	(1IV 18II 4I)
38·9/-101·9	(3IV 1III 14II 1I)
35·5/-100·2	(1III 20II 1I)
35·5/+101·0	(1IV 1V 2III 11II 7I)
35·8/+101·3	(3III 15II 5I)
36·5/+101·8	(1V 3IV 3III 9II 2I)
39·0/-102·5	(3IV 14II 4I)
39·1/-103·7	(1III 20II 1I)
23·8%-109·8	(2IV 18II)
30·5/+133·8	(1III 18II 5I)
36·8/+101·3	(2IV 1III 15II 3I)
36·8/+101·2	(3IV 14II 4I)
36·2/+101·9	(1VI 2IV 1III 12II 3I)
36·2/100·9	(2V 2III 12II 4I)
37·0/+102·1	A1 beginning. (1IV 2III 17II)
24·5/-108·8	(2VI 2IV 8II 2I)
24·4/-111·1	(3IV 16II)
27·2/-112·8	(1V 1IV 2III 13II 3I)
27·1/-112·8	(1VI 2III 15II 2I)
38·5/-102·4	(1VI 1III 13II 9I)
37·5/-103·0	

APPENDIX H.

Data of meiosis in the allotriploid (en P.veris x 4n P.elatior)

F141. (Slide 1) (2n P.veris x 4n P.elatior) (Started at corner 22·0/127·8)
(label to rt. Patholette 71)

22·9/104·9	(5III 7II 4I)
23·1/106·2	(7III 3II 6I)
23·2/105·1	(6III 5II 5I)
24·5/116·0	(7III 3II 6I)
25·8/135·1	(6III 4II 7I)
28·1/114·1	(1IV 5III 5II 4I)
27·9/121·1	(4III 8II 5I)
26·0/106·1	(3III 8II 8I)
32·0/136·1	(3III 9II 6I)
31·9/100·2	(4III 8II 5I)
32·0/107·1	(5III 6II 6I)
31·6/114·1	(1IV 4III 7II 3I)
31·9/114·1	(6III 5II 5I)
32·0/115·4	(7III 4II 4I)
33·5/115·0	(3III 10II 4I)
34·6/114·2	(6III 6II 3I)
36·9/135·2	(6III 6II 3I)
36·1/135·1	(5III 6II 6I)
36·0/139·4	(6III 6II 3I)
36·5/139·2	(2III 10II 7I)
37·5/110·0	(3III 10II 4I)
39·5/108·1	(2III 9II 9I)
40·0/107·1	(2III 11II 5I)
42·1/115·6	(4III 8II 5I)

APPENDIX H. (contd.)

F151n. (Slide 1) Patholette 71.

32·1/107·1	(6III 5II 5I)
32·0/107·2	(6III 7II 1I)
40·0/107·5	(5III 6II 6I)

F151n. (Slide 2. etc.)

45·9/137·8	(1III 12II 6I)
45·8/137·9	(3III 9II 6I)
45·9/137·8	(5III 5II 8I)
45·5/138·0	(1VI 6III 2II 5I)

G405(1) (2n P.veris x 4n P.elatior) Patholette 71 (label to rt.)

36·9/100·1	(8III 1II 7I)
35·0/110·8	(5III 6II 6I)
35·0/110·8	(5III 5II 8I)
32·1/117·0	(4III 7II 7I)
32·0/113·0	(1IV 1III 8II 10I)
31·0/99·9	(5III 6II 6I)
30·1/109·9	(5III 7II 4I)
27·1/101·1	(4III 7II 7I)
26·0/109·9	(5III 6II 6I)
26·0/109·9	(1IV 3III 6II 8I)
23·5/109·8	Central one of three - (4III 7II 7I)
23·5/110·0	(4III 8II 5I)
23·0/109·8	(4III 6II 9I)
23·1/109·8	(5III 5II 8I)
23·1/109·8	(4III 6II 9I)
23·0/109·8	(6III 4II 7I)
23·0/109·6	(3III 7II 10I)
23·0/109·1	(3III 9II 6I)
23·0/109·1	(3III 9II 6I)

BIBLIOGRAPHY

Benson, A.A., Bassham, J.A., Calvin, M., Goodale, T.C., Haas, V.A. and Stepka, W. (1950). "The path of carbon in photosynthesis, V. Paper chromatography and radioautography of the products". J. Amer. Chemical Soc. 72 (1); 1710.

Bruun, H.G. (1932). "Cytological studies in Primula". Symb. bot. Upsaliens. 1.

Cain, A.J. (1962). "The evolution of taxonomic principles". In "Microbial Classification" ed. by C. Ainsworth and P.H.A. Sneath 12th Symp. Soc. for Gen. Microbiology C.U.P.

Cain, A.J. and Harrison, G.A. (1958). "An analysis of the taxonomists' judgement of affinity". Proc. Zool. Soc. London. 131:85-98.

Chedda, H.R. and Harlan, J.R. (1962). "Mode of Chromosome association in Bothriochloa hybrids". Caryologia. 15: 461-476.

Chittenden, R.J. (1928). "Notes on species crosses in Primula, Godetia, Nemophilia, and Phacelia". J. Genetics 19; 285.

Clausen, J. (1958). "Gene systems regulating characters of ecological races and subspecies". Proc. Xth Int. Cong. Genetics, Vol.1.

Clausen, J. (1962). (Reprint of 1951 original). "Stages in the evolution of plant species". Hafner Publishing Co. Ithica, New York.

Clausen, J. and Heisey, W.M. (1958). "Experimental studies on the nature of species. IV. Genetic structure of ecological races". Carnegie Int. Washington. Publication 615.

Clifford, H.T. (1955). "Quantitative studies of hybridisation in wild plant populations". Ph.D. thesis, University of Durham.

Crosby, J.L. (1967). Personal communication.

Darlington, C.D. (1931). "Meiosis in diploid and tetraploid Primula sinensis". J. Genetics 24.

Darlington, C.D. (1932). "The control of the chromosome by the genotype and its bearing on some evolutionary problems". Amer. Nat. 66; 25-51.

Darlington, C.D. (1937). 2nd edition. "Recent advances in cytology". Churchill, London.

Davis, P.H. and Heywood, V.H. (1963). "Principles of Angiosperm taxonomy". Oliver and Boyd, London and Ediburgh.

Doroszewska, A. (1965). "Observations on Trollius chinensis x T. europaeus hybrids". Acta Societatis Botanicorum Poloniae vol. XXXIV nr.3.

Dunford, M.P. (1964). "A cytogenetic analysis of certain polyploids in Grindelia (Compositae)". Amer. J. Bot. 51; 49-58.

Fey, L. (1929). "Untesuchungen zur Phananalyse des Artebastardes Pr. varialilis Groupil (Pr. veris L em. Hudson x Pr. vulgaris Hudson), der Elternarten und von Primula elatior (L) Schreber". Doctorate dissertation; Zurich. Intitut für Allgemeine Botanik der Universitat Zurich II, Serie No.10.

Gajewski, W. (1957). "A cytogenetic study on the genus Gemn L". Monographia Botanicae, Vol.4; Warszawa.

Gajewski, W. (1959). "Evolution in the genus Gemn". Evolution 13; 378-388.

Grant, V. (1956). "The genetic structure of races and species in Gilia". Advances in Genetics VIII; 55-87.

Grant, V. (1967). "Linkage between morphology and viability in plant species". American Naturalist Vol.101, No.918; March-April.

Henderson, S. Alan. (1961). "The chromosomes of the British Tetrigidae (Orthoptera)". Chromosoma 12; 553-572.

Heslop-Harrison, J. (1962). "Purposes and procedures in the taxonomic treatment of higher organisms". In "Microbial Classification", ed. by G.C. Ainsworth and P.H.A. Sneath 12th Symp. Soc. General Microbiology. C.U.P.

Hull, David, L. (1967). "Certainty and circularity in evolutionary taxonomy". Evolution nr.1. Vol.21; pp.174-189.

Huskins, C.L. (1929). "Primula juliae and its hybrids with the primrose and the oxlip". Journal of the Royal Horticultural Society. The Fourth Primula Conference.

Levin, Donald A. (1966). "The Phlox pilosa complex; crossing and chromosome relationships". Brittonia 18 (2); 142-162.

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

Marsden-Jones, E.M. (1930). "The genetics of Geum intermedium Willd. H and Ehrh., and its backcrosses". Journal of Genetics XXIII; 3

Mayr, E. (1963). "Animal species and evolution". London, Oxford Univ. Press.

Mentzel, Margaret Y. (1962). "Pachytene chromosomes of the intergeneric hybrid Lycopersiconesculentum x Solanum lycopersicoides". American Journal of Botany 49 (6), pp.605-614.

Morrison, J.W. and Rajharty, T. (1960a). "Frequency of quadrivalents in autotetraploid plants". Nature; Vol.187; nr. 4736; August 6th.

Morrison, J.W. and Rajharty, T. (1960b). "Chromosome behaviour in autotetraploids". Chromosoma 11.

Newman, L.J. (1966). "Bridge and fragment aberrations in Podophyllum peltatum". Genetics 53; 1: 55-63.

Newton, W.C.F. and Pellew, C. (1929). "Primula kewensis and its derivatives". Journal of Genetics 20; pp.405-467.

Pax, F. (1889). in "Engl. Bot. Jahrb.", X, 177.

Pearson, P.L. (1965). Personal communication.

Percival, Mary S. (1961). "Types of nectar in Angiosperms". New Phytologist 60; 235-281.

Peto, F.H. (1933). "The cytology of certain intergeneric hybrids between Festuca and Lolium". J. Genetics XXVIII.

Prazmo, W. (1965). "Cytogenetic studies on the genus Aquilegia III Inheritance of the traits distinguishing different complexes in the genus Aquilegia". Acta Societatis Botanicorum Poloniae. Vol.XXXIV - nr.3.

Riley, R. (1960). "The diploidisation of polyploid wheat". Heredity 15 (4).

Riley, R. (1966). "Genetics and the regulation of meiotic chromosome behaviour". Science Progress, 54; No.214; 193-207.

Roseweir, J. and Rees, H. (1962). "Fertility and chromosome pairing in autotetraploid rye". Nature, 195; No.4837 (July 14th).

Skalinska, Maria (1964). "Studies in the origin of some European species of Aquilegia". Acta Biologica Cracoviensia; Series Botanica Vol.VII, pp.1-24.

Smith, W.W. (1933). "Some aspects of the bearing of cytology on taxonomy". Proc. Linn. Soc. 145; 151-181.

Smith, W.W. and Forrest, G. (1928). "The sections of the genus Primula". Notes Roy. Bot. Soc. Gard. Edinburgh, 16; 1-50.

Smith, W.W. and Fletcher, H.R. (1947). "The genus Primula: section Vernales Pax". Trans. Bot. Soc. Edinb. 34; 402.

Sneath, P.H.A. (1962). "The Construction of Taxonomic Groups". In "Microbial Classification" ed. by C. Ainsworth and P.H.A. Sneath 12th Symp. Soc. for General Microbiology. C.U.P.

Sneath, P.H.A. and Sokal, R.R. (1962). "Numerical Taxonomy". Nature 193; 855-860.

Snow, Richard. (1963). "Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations". Stain Technology 38(1); 9-13.

Sokal, Robert R. (1963). "The Principles and Practice of Numerical Taxonomy". Taxon. XII, 5; 198.

Solbrig, O.T. (1966). "Evolution and Systematics". Macmillan.

Sporne, K.R. (1956). "The phylogenetic classification of the angiosperms". Biol. Rev., 31; 1-29.

Stebbins, G.L. (1938). "Cytogenetic studies in Paeonia.II. The cytology of the diploid species and hybrids". Genetics 23; 1: pp.83-110.

Stebbins, G.L. (1947). "Types of polyploid; their classification and significance". Advances in Genetics 1; 403-429.

Stebbins, G.L. (1950). "Variation and Evolution in Plants". Columbia.

Stebbins, G.L. (1958). "The inviability, weakness and sterility of interspecific hybrids". Advances in Genetics IX.

Stebbins, G.L. (1966). "Processes of organic evolution". Prentice Hall.

Stevens, S.G. (1942). "Colchicine produced polyploids in Gossypium. I. An autotetraploid Asiatic cotton and certain of its hybrids with diploid spp." J. Genet. 44; 272-295.

Swain, T. (1963). "Chemical Plant Taxonomy". Academic Press: London.

Swanson, C.P. (1960). "Cytology and Cytogenetics". Macmillan.

Swietlinska, Zofia. (1963). "Cytogenetic relationships among Rumex acetosa, Rumex arifolius and Rumex thyrsiflorus". Acta Societatas Botanicorum Poloniae, Vol.XXXII, Nr.2.

Valentine, D.H. (1947). "Studies in British Primulas. I. Hybridisation between Primrose and Oxlip (P. vulgaris Huds., and P. elatior Shreber)". New Phytologist 46; 229.

Valentine, D.H. (1948). "Studies in British Primulas. II. Ecology and taxonomy of Primrose and Oxlip. (P. vulgaris Huds. and P. elatior Scheber)". New Phytologist 47; 111-130.

Valentine, D.H. (1951). "Geographical distribution and isolation in some British ecospecies". in "The study of the distribution of British Plants". ed. by J.E. Lonsley.

Valentine, D.H. (1952). "Studies in British Primulas. III. Hybridisation between P. elatior (L) Hill and P. veris L.". New Phytologist 50; 383.

Valentine, D.H. (1953). "Evolutionary aspects of species differences in Primula". Symp. Soc. Exp. Biol 7; 146.

Valentine, D.H. (1954). "Seed incompatibility". Proc. 8th Int. Bot. Congr. Section 9; 170.

Valentine, D.H. (1955). "Studies in British Primulas. IV. Hybridisation between P. vulgaris Huds. and P. veris L. New Phytologist 54; 70.

Valentine, D.H. (1956). "Studies in British Primulas. V. The inheritance of seed incompatibility". New Phytologist 55; 289.

Valentine, D.H. (1961). "Evolution in the genus Primula". in "A Darwin Centenary" ed. P.J. Wanstell, pub. T. Buncle and Son, Arbroath.

Valentine, D.H. (1962). "Sections and genera in the Primulaceae. Taxon, XI nr.3.

Valentine, D.H. (1963). "Patterns of speciation in higher plants". Archivum Societatas Zoologicae Fennicae 'Vanamo'.

Valentine, D.H. (1965). "Recent advances in cytotaxonomy in Europe". Rev. Romn. Biol. (Bot.) 10; 19-25.

Valentine, D.H. and Woodell, S.R.J. (1963). "Studies in British Primulas X. Seed incompatibility in intraspecific and interspecific crosses at diploid and tetraploid levels". New Phytologist 62; 125-143.

Walters, S.M. (1963). "Methods of Classical Plant Taxonomy". in T. Swain (ed.), "Chemical Plant Taxonomy", London, Academic Press. pp.1-16.

Wendelbo, P. (1961). "Studies in Primulaceae II". Arbok fur Universitetet i Bergen, No.11.

Wherry, E.T. (1955). "The Genus Phlox". Morris Arb. Monogr. 3, Philadelphia.

Woodell, S.R.J. (1960). "Studies in British Primulas. VII. Development of normal seed and of hybrid seed from reciprocal crosses between P. vulgaris Huds. and P. veris L". New Phytologust 59, pp.320-313.

Woodell, S.R.J. (1965). "Natural hybridisation between the cowslip (Primula veris L.) and the primrose (P. vulgaris Huds.) in Britain". Watsonia 6; pp.190-202. part 3.