

Investigation into the relationships and
ancestry of Vulpia and Festuca

by

C.M. Barker

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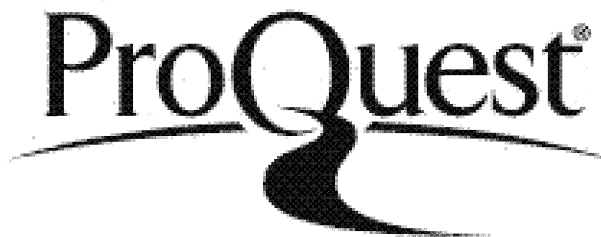
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STATEMENT

The contents of this thesis are based on work conducted by the author in the Department of Botany, University of Leicester, during the period October 1976 - June 1979.

All work recorded in this thesis is original, unless otherwise acknowledged in the text or by references.

SignedCaroline Barker.....

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CHAPTER 1

THE TAXONOMIC STUDY OF GRASSES

1.1 The importance of grass biosystematics

One of the aims of biosystematics is to interpret data from studies of geographical variation, cytology, breeding behaviour and ecological adaptation in terms of relationships between taxa. The study of evolutionary relationships in the Poaceae (Gramineae) has been of particular interest because of the economic importance of many members of this family. Plant breeders have been especially interested in determining the wild relatives of wheat, barley, oats, rye, maize, millet, rice and sugar cane, because the closely related species may have characters which could be introduced into the agricultural types to improve their yield and quality.

The agricultural importance of grasses has inspired their taxonomic study, but it is only relatively recently that evolutionary relationships between taxa have been detected, because for various reasons it is a difficult family to study.

The family Poaceae has an estimated 620 genera and 10,000 species (Willis, 1973). It has the third largest number of genera after the Compositae and the Orchidaceae and has the fourth largest number of species after the latter two families and the Leguminosae. Grasses occupy an enormous variety of habitats throughout the world and during their evolutionary history have probably undergone extensive parallel and convergent evolution (Stebbins & Crampton, 1961). The structure of the grass flower is so reduced and condensed and the leaves are superficially of such a uniform type that characters of grass morphology are limited in number and interpretative value.

The early classical taxonomic studies of the Poaceae, based purely on morphological characters, resulted in a highly artificial classification. However, the reduced floral structure has encouraged grass taxonomists to find other features to differentiate between taxa. Most of the additional taxonomic characters are cryptic (microscopic) from the study of morphology, anatomy, cytology and chemistry.

The aim of most plant taxonomists is to produce a natural classification, based upon as many characters as possible, which many workers consider will reflect the evolutionary relationships. However, phenetic relationships, based upon perceived similarities between plants may not necessarily coincide with phylogenetic relationships (Smith, 1976). More conclusive evidence concerning evolutionary relationships between genera and lower taxa can come from hybridisation studies. This topic will be dealt with in the next chapter.

The aim of the present study is to determine the relationships between red fescue, Festuca rubra L. sensu lato, an important amenity and forage grass of Britain and West Europe, and species of the annual genus Vulpia C.C. Gmelin, as well as further information on relationships within the genus Vulpia.

1.2 History of grass systematics

In the first edition of Species Plantarum, Linnaeus (1753) listed 40 grass genera (Gould, 1968). The so-called sexual system of classification developed by Linnaeus was based on the number and arrangement of stamens and carpels. This system sometimes resulted in the grouping together of very dissimilar plants or in separating those plants which shared many other similar features. For the grasses, Linnaeus listed 28 genera under Triandria-Digynia, but Anthoxanthum was placed in Diandria-Digynia, Oryza was in Hexandria-Digynia and Andropogon, Holcus

and Aegilops were placed in Polygamia-Monoecia.

During the nineteenth century, there was a general shift in the objectives of systematics to the grouping together of morphologically similar plants. Robert Brown (1810) was the first to understand the true nature of the grass spikelet and to recognise it as a reduced inflorescence branch. This enabled him to perceive certain fundamental characters in the spikelet which he used as the basis of the "series" Panicaceae and Poaceae. Bentham (1881) retained Brown's subfamilies and divided the Panicaceae into six tribes and the Poaceae into seven tribes and further divided some of the tribes into subtribes. Later, Hackel (1887) adopted a similar classification of tribes, but dropped the two "series".

The systems of grass classification based on morphological characters have been revised in the light of many, new, cryptic characters which have been useful at all taxonomic levels. The consensus of modern opinion recognises the following six subfamilies in the family Poaceae (Clayton, 1978): Bambusoideae, Centostecoideae, Arundinoideae, Chloridoideae, Panicoideae, Pooideae (Festucoideae).

Stebbins and Crampton (1961) presented a list of taxonomic characters which have been useful in the classification of grass genera into tribes and tribes into subfamilies. Prat (1960) added to Stebbins' and Crampton's list and Auquier (1963) enlarged on Prat's list.

i. Morphological and anatomical characters

The first with the new approach to grass systematics were the anatomists; later, cytological characters were realised to be of importance.

The anatomical and histological features of grass leaves became the subject of research by many botanists around 1900. Duval-Jouve

(1875) was the first to use grass leaf anatomy in systematics. The character he used was the position of the bands of bulliform cells in relation to the veins as seen in transverse sections of the leaf. Grob (1896) listed species with bicellular hairs and Schwendener (1890) discussed the nature of the mestome and the parenchyma sheaths that surround the vascular bundle. These workers suggested the value of anatomy as a taxonomic tool but it was not until later that it was used extensively.

Prat (1932, 1936) was the first to recognise that the epidermal structure of leaves also provides good taxonomic characters. The arrangement of internal tissues of grass leaf blades was classified into two distinct types by Avdulov (1931). Prat (1936) named these the "festucoid" and "panicoid" type of anatomy. Stebbins (1956) added two more types, "bambusoid" and "chloridoid" to the two recognised by Avdulov and Prat. Brown (1958) investigated the leaf anatomy of 101 species in 72 genera and found six basic types of tissue arrangement, which could be correlated with six major taxonomic groups: bambusoid, pooid (festucoid), arundoid, panicoid, aristidoid and chloridoid.

Tateoka et al. (1959) examined the microhairs on the leaf epidermis of 238 grass species and reconfirmed the importance of microhairs in systematics at the subfamily and tribal levels. Metcalfe (1960) described in detail those characters of the epidermis of 345 genera and also those of internal anatomy, which can be of diagnostic value in grass systematics. Apart from a comparatively small number of taxa with mixed characters, Metcalfe (1960) found that grasses can be divided into pooid and panicoid groups on the basis of leaf structure. Whereas the panicoid grasses fall easily into groups which appear to be natural, Metcalfe pointed out that leaf structure is of limited value in grouping the tribes of pooid grasses. Ellis (1976) has recently attempted to introduce

uniform standards to the description of grass leaf blades.

Lodicules, the scales situated at the base of the ovary in grasses, are thought to correspond to the inner whorl of the perianth (the outer whorl being absent) (Arber, 1934). Krause (1909) recognised that lodicules have some systematic value but it was not until relatively recently that they have been used as indicators of taxonomic relationships. Stebbins (1956) recognised four types of lodicule based on their number, shape and vascular traces. Other studies on the morphology of lodicules have been made by Reeder and Ellington (1960), Tateoka (1960), Decker (1964), Hsu (1965) and Jirásek and Josífová (1968).

Takagi (1964) examined the lodicules of various bamboo species and discovered microhairs on the lodicule epidermis. Tateoka and Takagi (1967) examined the lodicules of 288 species of grasses representing 146 genera and found that the Pooideae are completely lacking in microhairs on the lodicules whereas they are abundant on the lodicules of the Bambusoideae and less common on lodicules of the other subfamilies.

Van Tiegham (1897) discovered two types of grass embryo, panicoid and pooid. He took longitudinal, sagittal sections of the embryo and looked at the presence or absence of the scutellum and the course of the vascular trace. Prat (1932) cited Van Tiegham's embryology in his discussion of the grass system, and in 1936 (Prat, 1936) used it as one of the contrasting characters of pooid and panicoid grass types. Reeder (1946, 1953) verified the importance of embryology in grass systematics. Reeder (1961) made histological investigations of embryos of nearly 400 species of grasses, representing more than 175 genera. Reeder (1961) recognised six groups of grasses and in most cases the validity of these groups is supported by evidence from leaf anatomy, histology and cytology.

Other workers have recently found new characters to delimit the grasses at the subfamily level. Reeder and Maltzahn (1953) found two different types of root hair development in grasses which could be correlated with the differences found by the study of embryo structure, cytology and leaf anatomy. Row and Reeder (1957) found that the alternation of long and short cells (poooid) versus equal-sized cells (panicoid) in root hairs was the most reliable character to use. Brown, Heimsch and Emery (1957) examined the organisation of the grass shoot apex and again found two types corresponding to the poooid and panicoid groups of grasses. Cheadle (1960) also found a difference in specialisation of vessels for the two subfamilies. Brown, Harris and Graham (1959) examined the stem internodes of 133 species of grasses from 80 genera. They reported that while about 93% of the poooid grasses examined had hollow internodes, the number of grasses with solid or semi-solid internodes in the tribes Eragrostoidae and the Panicoideae ranged from 49 to 100%. Brown, Pratt and Mobley (1959) examined the development of the meristematic swellings (pulvini) at the base of the culm internode and at the base of the sheath. Typical poooid stems with hollow internodes were found to lack culm pulvini but had well-developed sheath pulvini. In contrast, panicoid and eragrostoid grasses with solid and semi-solid stems tended to have well-developed culm pulvini and no sheath pulvini.

Morphological and anatomical characters have also been used in classification at the genus and species level; epidermal characters have been especially useful. Sørensen (1953) employed the structure and size of the epidermal cells with success in his revision of the Greenland species of Puccinellia. Church (1949) has shown that the possession of intercostal long cells with smooth walls serves to distinguish Glyceria from Puccinellia and Torreyochloa in which the corresponding cells have ripple walls. Björkman (1960) described an epidermal network (trichodium net) in the lemma of Agrostis species.

The trichodium net was reported for Calamagrostis tenella by Paunero (1953) but in the rest of the genus the network is absent. Björkman (1960) used this as part of the evidence for transferring this species to Agrostis.

Harz (1880) was the first to recognise that different types of starch grains had a systematic value and showed that starch grains of the grass endosperm could be divided into three types. Krause (1909) replaced these by a simpler division into two types, simple and compound. Avdulov (1931) reviewed the features of starch grains appearing in the preceding papers and compared them with his cytological findings. Avdulov found that the features of starch grains are not always exactly correlated in the subfamilies with other basic systematic characters. Tateoka (1962) examined the endosperm starch grains of 766 grass species belonging to 244 genera and categorised the starch grains into four types but found them useful in systematics for only a few cases.

Brown and Emery (1957) found that the character of persistent nucleoli is of systematic value. They examined plants representing 45 species from 39 genera and found that all root tips of grasses of the subfamily Panicoideae have persistent nucleoli in some, but not all, cells and that the Pooideae never have persistent nucleoli in any of the cells.

ii. Cytological characters

The contributions made by cytology to taxonomy form an outstanding feature of the past few decades. Karyotypes and chromosome behaviour at meiosis may be used both for comparison and for evolutionary interpretation. Three major characteristics of karyotypes are usually observed and compared: chromosome number, gross morphology and staining properties.

Cytological investigations of the Poaceae began about the 1920s when the chromosome numbers of the cereals and their close relatives were counted. Avdulov (1931) observed chromosomes in the root tips of 232 grass species distributed among nearly all the recognised tribes. On the basis of chromosome number and size he divided the grass family into three main groups, and he found that these groups were closely associated with the expression of the following characters: the shape and position of the first seedling leaf, the organisation of the resting nucleus, the starch grains of the seeds, geographical distribution and especially leaf morphology and anatomy. The three groups were the Phragmitiformes, characterised by the basic chromosome number $x = 12$ and small chromosomes; the Festuciformes, having $x = 7$ (rarely 6 or 5) and large chromosomes; and the Sacchariferae, with $x = 10$ or 9 and small chromosomes. Tateoka (1960) reported that the number of grass species whose chromosome numbers were determined by Avdulov and preceding investigators totalled 370 species.

Carnahan and Hill (1961) listed the chromosome numbers of more than 1,550 grass species. Their analysis of these species shows that over 90% fall into one of two major groups: the tropical or sub-tropical species with small chromosomes have a base number $x = 10$ or simple derivatives such as nine or twelve; and those species with a mainly temperate distribution have larger chromosomes and the base number is usually seven.

The lowest chromosome number reported in the Poaceae is $2n = 8$, as found in Milium scabrum L.C.M. Richard (Milieae), Airopsis tenella (Cav.) Ascherson & Graebner (Aveneae), Holcus gayanus Boiss. (Aveneae), Iseilema laxum Hack. (Andropogoneae) and a few other species. Unusually high numbers have been reported for Alopecurus alpinus Sm. and A. glaucus Less. (= A. alpinus subsp. glaucus (Less.) Hultén) (Aveneae) $2n = 130$, species of Andropogon (Andropogoneae) $2n = 234, 239$,

Calamagrostis crassiglumis Thunb. (Aveneae) $2n = 140$, and many others.

In many cases, cytological studies have helped to increase the systematic understanding of some controversial genera or have exposed systematic problems which need to be studied more deeply. Stebbins and Löve (1941) found that Distichlis is cytologically quite dissimilar from typical members of the Poeae to which it was usually ascribed on the basis of morphology. Stebbins and Löve (1941) also examined seven species of Melica which were all found to have $2n = 18$; Melica was previously treated as congeneric with Schizachne which has $x = 10$ and smaller chromosomes. The members of the tribe Pappophoreae had been treated as belonging to Pooideae; however their chromosome numbers $x = 9$ or 10 and small size, reported by various authors suggested their affinity to Eragrostoideae. Cáceres (1958) also demonstrated the close relationship of Pappophoreae to eragrostoid grasses by examining the anatomical characters of the leaf and Reeder (1957) reached the same conclusion by studies of embryology.

Avdulov (1931) found that the chromosome constitution of Brachypodium (small chromosomes and variable base number) is strikingly different from those of other genera of the tribes Triticeae or Poeae, which have a base number of seven and large chromosomes. Tateoka (1956) suggested that Brachypodium is phylogenetically different from the other genera of Triticeae and should be placed in a tribe of its own. Melderis (1978) suggested that Brachypodium serpentini C.E. Hubbard could not be placed in Brachypodium because it has pooid characters and could not either be placed in Agropyron or Elymus. Melderis (1978) proposed a new genus Festucopsis belonging to the tribe Triticeae, containing two species, F. serpentini (C.E. Hubbard) Melderis and F. sancta (Janka) Melderis. I.H. Robertson (pers. comm. 1979) later found that the chromosomes of F. serpentini were large like those of the other genera in the tribes Triticeae and Poeae.

There are many other examples where unexpected chromosome number or size revealed in some taxa, have led to intensive investigations of their other systematic characters.

Of the grass species for which the chromosome number was known, Carnahan and Hill (1961) estimated that about 80% were polyploid, with 7% of these aneuploid. Stebbins (1950) estimated that the percentage of polyploid species in the flowering plants in general is 30 - 35% and Grant (1963) estimated 47%. Thus grasses have about twice as many polyploids as the average for angiosperms.

Since the trend of evolution from diploid to polyploid is predominantly irreversible (although de Wet (1971) cited some examples where autotetraploids have reverted to diploids) polyploid complexes are particularly useful for analysing problems of plant geography and phylogeny. The relative abundance of polyploids as compared with their diploid ancestors in a polyploid complex increases with its age. On this basis Stebbins (1971) recognised five stages of maturity of a polyploid complex: initial, young, mature, declining and relictual. Stebbins (1971) gave the genus Aegilops as an example of a young polyploid complex. This genus has been studied in detail by Zohary (1965) as well as others. The genome of diploid A. umbellulata Zhuk. is found in seven distinct tetraploid species. The second genome in these tetraploids is derived from ancestral diploids related to one of three different species complexes of Aegilops: A. caudata L. (= A. dichasians (Zhuk.) Humphries), A. comosa Sibth. & Sm. and A. uniaristata; ranges of all these overlap with that of A. umbellulata. However, two of the tetraploid species have spread far beyond the limits of all the diploids.

Dactylis is an example of a mature polyploid complex (Stebbins, 1956). The diploid species constitute only a small fraction of the genus, and most diploids are very restricted in their distribution.

Over the great bulk of its geographical distribution Dactylis is represented by tetraploid strains; some hexaploids are found in North Africa (Borrill, 1976). The Festuca rubra aggregate can also be considered as a mature polyploid complex. Of the 23 species (Markgraf-Dannenberg, 1980) four are diploids and their distribution is restricted to the Alps and a few other mountainous areas in Europe. The other species range in ploidy level from tetraploid to decaploid. Hexaploid F. rubra is the most widely distributed species, growing throughout most of the North Temperate zone.

The basic haploid complement varies in the shape, length and volume of the chromosomes, as well as the number and size of satellites and position of secondary constrictions. Comparison of karyotypes is profitable when the chromosomes are large enough to be studied in detail. The constant position of the centromere allows chromosomes to be characterised by the relative lengths of the arms. The classification of different types has been described by Levan et al. (1965). Measurement of chromosome size and arm ratios is subject to many sources of error, both biological and technical; these difficulties have been summarised by Bentzer et al. (1971).

In general, changes in symmetry and shape are due to various structural alterations, i.e. translocations, inversions, duplications, deletions, fissions and fusions. The ways by which such changes have arisen in a particular karyotype gives valuable information on the origin of the group concerned. However, structural changes may occur which are not reflected in the morphology or number of the chromosomes. The order of genes in the chromosome arms may be shifted by inversions, for example, a feature of considerable evolutionary importance since it may lead to intersterility between plants without any accompanying phenotypic change. Stebbins (1945) has called this type of mismatching of parental chromosomes cryptic structural hybridity.

It has proved possible to work out evolutionary trends of karyotype features in some groups of plants. In general, there appears to be a correlation between asymmetry in the karyotype and evolutionary specialisation of the plant as in the genus Crepis (Babcock, 1947). In the Poaceae, tribe Triticeae, most of the species have relatively symmetrical karyotypes, but in certain genera of annuals such as Aegilops (Kihara, 1940) increasing karyotype asymmetry is associated with increasing specialisation in reproductive structures (Stebbins, 1958). There are, however, exceptions to this rule, symmetrical karyotypes being found in many groups regarded as highly evolved or specialised morphologically. The direction of chromosome change has been investigated recently by Jones (1978). From the studies of centric fusion in various genera (Gibasis, Cymbispatha, Zebrina, Nothoscordum, Haplopappus and others) and from the fact that most primitive angiosperms have small asymmetrical chromosomes, Jones (1978) suggests that centric fusion between acrocentric or telocentric chromosomes may have been very important in karyotype evolution and that asymmetrical chromosomes may be more primitive than metacentric chromosomes.

Various stains have been used to colour plant chromosomes, notably Feulgen reagent. Conventionally stained chromosomes do not have many characters which can be used to distinguish them within a complement, and cytologists have tried to devise staining techniques for chromosome differentiation.

Heitz (1935) recognised the presence of two types of chromatin in the chromosomes, namely euchromatin and heterochromatin. Heterochromatin can be distinguished from euchromatin because it maintains its metaphase condensation at interphase when it takes the form of heteropycnotic chromocentres. Darlington and LaCour (1940) differentially stained the chromosomes of Trillium by cold treating the living plant. The chromosomes then appear thin and understained at certain specific regions which correspond to the location of heterochromatin (Baumann, 1971).

The most effective differential stains are Giemsa, first used with mouse chromosomes and subsequently widely employed in human and animal cytogenetics, and quinacrine dyes which fluoresce when viewed under ultraviolet light. With the adaptation of these techniques to plant material, Giemsa staining especially has now been applied to a range of taxa (Vosa, 1975). In Trillium grandiflorum Salis., Scilla siberica Haw. and others, Giemsa stained chromosome segments have been shown to be identical with the heterochromatic regions revealed by cold treatment (Schweizer, 1973).

The differential staining of chromosomes with Giemsa provides new chromosome characters in the size and position of the stained bands. Giemsa staining techniques have been used to produce banding in the somatic metaphase chromosomes of several species of the Poaceae. These include rye (Sarma & Natarajan, 1973; Verma & Rees, 1974; Gill & Kimber, 1974) where hitherto indistinguishable chromosomes can now be identified, including the rye chromosomes in Triticale (Merker, 1973; Bennett & Smith, 1975). In wheat, banding has been used in the search for the progenitors of the hexaploid Triticum aestivum L. (Gill & Kimber, 1974; Natarajan & Sarma, 1974; Hadlaczky & Belea, 1975), whilst Hadlaczky and Kalman (1975) used the banding pattern in maize to identify chromosomes from different stocks and hybrids. Banded karyotypes have also been made of barley (Linde-Laursen, 1975; Vosa, 1976), Avena (Yen & Fillion, 1976) and Lolium temulentum L. (Thomas, 1977). Banded karyotypes of Agropyron and Festuca are reported by Kimber et al. (1975) but the species used and the observations are not mentioned.

iii. Chemical characters

Plant taxa exhibit chemical variation which, like any other facet of their diversity, is a source of new characters or information useful in taxonomy.

Beval and Cugnac (1941) studied the variation in the water-soluble fructans in the caryopses of a range of grass genera. Certain genera in the tribe Triticeae (Hordeum, Triticum, Secale and Aegilops) possess indistinguishable fructans, which are referred to as levosine, and other triticoid genera, namely Agropyron and Elymus, have very similar fructans. Festuca and Lolium (tribe Poeae) were found to have a fructan like phlein, from Phleum, Phalaris and Ammophila (Aveneae s.l.) (Smith, 1969). Though the affinities of Bromus would have been expected to lie more with Poeae than with Triticeae, the fructans of this genus in fact resemble those of the latter tribe (Smith, 1976).

Most of the recent biochemical work in grasses has been carried out using proteins, although grasses also have secondary plant products such as alkaloids.

Serological techniques have been used to provide one of several lines of new taxonomic evidence in the revision of the genus Bromus (Smith, 1971, 1972). Infrageneric taxa such as sections or sub-sections in Bromus s.l., recognised on the basis of morphology, geography and interfertility are sometimes elevated to the rank of genera (e.g. Anisantha, Ceratochloa, Trisetobromus). Between all these groups there is a strong morphological similarity. When Smith found that serological data also showed similarities between these groups, but not between Bromus s.l. and Festuca or Brachypodium, he decided that the broader generic concept should be adopted and that the infrageneric units should be regarded as sections.

During the comparisons of Bromus species with other grass species, Smith (1976) recognised a serological gulf between Bromus and most other genera. The exception to this finding was Boissiera, a monotypic genus of annual plants, long supposed to approach Bromus in certain morphological characters. Serological data confirmed the close relationship to Bromus and Smith (1969) has incorporated this species into Bromus as B. pumilio (Trin.) P.M. Smith.

Chromatography and electrophoresis have proved to be very suitable techniques for taxonomic surveys, as both permit the screening of many samples in a short time. Electrophoresis of storage proteins in wheat shows a close correlation with genomic constitution (Johnson & Hall, 1965). The protein patterns in the acrylamide gel show that amphidiploid wheats Triticum aestivum L. (AA BB DD) and T. dicoccum Shrank (AA BB) possess almost all the proteins of the A genome as shown in the diploid T. monococcum L. (AA). They also share proteins of the B genome, which is thought to be an ancestral type of Aegilops speltoides Tausch. The D genome of the hexaploid wheats is usually thought, mainly on morphological and cytological evidence, to have come from Aegilops squarrosa L. By disc electrophoresis, Johnson (1972) has demonstrated the high probability of the ancestry of T. aestivum from A. squarrosa (DD) and T. dicoccum, by mixing their proteins artificially. The mixture has the same electrophoretic properties as extracts from T. aestivum. From these results and from other work it has been found that chemical characters of hybrids and hybrid derivatives, such as amphidiploids, are mostly additive, i.e. they possess the sum of the compounds present in both parents. Schwartz (1960) found that maize hybrids showed both complementation and the presence of new enzymes which neither parent produces.

More chemotaxonomic investigations are needed as they have been rewarding at all hierarchical levels and they may therefore provide new insights into evolutionary relationships.

CHAPTER 2

HYBRIDISATION AS A GUIDE TO SPECIES RELATIONSHIPS

2.1 Crossability

Natural interspecific hybrids are more numerous in some families than in others. The Orchidaceae and the Poaceae have given rise to particularly large numbers of natural hybrids, which are well documented because of their economic value as well as their intrinsic interest.

Ullman (1936) tabulated most of the grass hybrids known up to 1936. He reported 74 naturally occurring and 64 artificial hybrids. Later, Myers (1947) listed over 200 interspecific and intergeneric hybrids known in forage grasses; of these 93 were naturally occurring and the others were produced artificially. Carnahan and Hill (1961) listed 256 interspecific and 95 intergeneric hybrids, composed mostly of artificial hybrids but also including a number of sterile natural hybrids. A more recent survey of grass hybrids (Knobloch, 1968) listed more than 2,400 hybrids, both interspecific and intergeneric. Unfortunately this checklist does not differentiate between naturally occurring and artificial hybrids.

The relative abundance of natural interspecific hybrids in the Poaceae appears to be related to the frequent occurrence of different species growing together in dense stands, the production of large amounts of wind-borne pollen and the existence of poor or incomplete genetic isolating systems. The survival and successful establishment of many of these new hybrids is associated with their perennial habit and effective means of vegetative reproduction. Also, the high frequency of polyploidy in the family has provided a means of fixing and spreading hybrid combinations.

At the supra-specific level hybrids are much less frequent and, because of this, it has been suggested that the occurrence of hybrids between species of different genera indicates that the genera should be taxonomically combined. The great majority of intergeneric hybrids involve obviously closely related genera. In the Poaceae all natural hybrids so far described involve genera within a single tribe, although successful inter-tribal crosses have been carried out artificially (i.e. Bromus x Festuca) (Stace, 1975).

Much early work involving herbage grass hybridisation was carried out at the Welsh Plant Breeding Station (W.P.B.S.), Aberystwyth, by Jenkin. Incompability systems in grasses have been studied by Lundqvist (1961, 1962, 1968).

Species are normally prevented from crossing by isolating mechanisms, the breakdown of which allows hybridisation to take place. Hybridisation can be a sign that the distinctness which once existed between two species is breaking down, or in others that the two species have not yet become sufficiently distinct (Stace, 1975). In yet other cases hybridisation occurs between normally well-isolated species and does not lead to the breakdown of specific boundaries. Although in any one group the degree of morphological similarity is likely to be proportional to the interbreedability, overall this is not so. In many groups fully fertile hybrids occur in nature between taxa of vastly different appearance, and in others there are strong sterility barriers between taxa which are hardly, if at all, morphologically separable (Stace, 1975). Therefore, species cannot be delimited on the basis of intersterility alone.

Isolating mechanisms are of many different sorts and have been classified by authors who have cited examples of species apparently isolated by each of the various categories (i.e. Dobzhansky, 1937;

Stebbins, 1950; Dobzhansky, 1951; Baker, 1951; Riley, 1952; Davis & Heywood, 1963; Solbrig, 1968; Levin, 1971; and Stace, 1975). Isolating mechanisms are said to be of two main sorts, external and internal. Barriers to hybridisation up to the formation of a hybrid zygote are termed prezygotic, those mechanisms preventing a viable hybrid progeny from the zygote, are termed postzygotic barriers. In nature, most species are isolated by a combination of several sorts of barrier, often some internal and some external.

External isolating mechanisms depending on differences in ecology, geography, flowering season, the time of flowering within the day, type of pollinator, and breeding behaviour can often be overcome easily under artificial conditions. Geographical and ecological barriers and differences in flowering season can be overcome by cultivation of the species in greenhouses with the necessary soil, climate or photoperiodic cycle provided. Breeding behavioural differences can be overcome by emasculation and manual pollination. Temporal isolation offers few problems as pollen usually remains viable for a few hours and in some species can remain viable for many months when frozen (Santamour, 1972).

Interspecific incompatibility acts as an internal breeding barrier between sympatric species. de Nettancourt (1977) has defined interspecific incompatibility as any of the post-pollination processes preventing, through an absence of pollen germination or an abnormal behaviour of pollen tubes, the formation of hybrid zygotes. Many cases are known where the self-compatible species can hybridize with related self-incompatible species when the former are the female parents, but not when they are the male parents. This phenomenon has been called unilateral incompatibility (Harrison & Darby, 1955) and is thought by Lewis and Crowe (1958) to be the most basic and common manifestation of

interspecific incompatibility. Nordenskiöld and Quincke (cited in Lewis & Crowe, 1958) have found many examples of unilateral incompatibility among the Poaceae. The main exceptions to this directional rule concern self-compatible species only recently mutated ~~from~~ self-incompatible ones (Lewis & Crowe, 1958). However, Abdalla and Hermesen (1972) and Hogenboom (1972, 1973, 1975) note that unilateral incompatibility also occurs between self-compatible species in Antirrhinum, Lycopersicum and Nicotiana, and also between populations of self-incompatible plants. Hogenboom also refers to various cases where unilateral incompatibility has been observed between self-compatible species as maternal parents and self-incompatible species as the pollen source.

In other cases of interspecific incompatibility the ploidy level of the parents is of importance. Watkins (1932) found that if the normal ploidy ratio of pollen: style (1:2) is lowered (e.g. 2:2 as in a diploid female x tetraploid male) the pollen tubes are unsuccessful in negotiating the style, but are reasonably successful in the reciprocal cross (which gives 1:4). This phenomenon has been demonstrated in several genera. Nevertheless there are exceptions as in Prunus, where hybrids between diploid and tetraploid cherries are much more easily obtained if the diploid is used as the female (Crane & Lawrence, 1956).

There are many genera known where it has been found easier to produce hybrids by crossing polyploids than by crossing diploids. Fagerlind (1937) found that both Galium mollugo L. and G. verum L. are either diploid or tetraploid: at the diploid level they are intersterile yet at the tetraploid level fertile hybrids are formed. Grant (1965) also found hybridisation between tetraploid species or races of Lotus much easier than that between diploids. This has led to the technique of obtaining crosses impossible or difficult at the diploid level by using natural or artificially induced tetraploids. In some cases however,

the reverse is true; Nordenskiöld (1956) found crossing more difficult and the fertility of the hybrids lower in Luzula the higher the ploidy level.

Experiments of artificial hybridisation are obviously limited by time and expense and because of this only a few different genotypes of the parental species are usually used for crossing. However in the wild, when different species are growing closely together, the different combinations of genotypes available to cross must be very large. This massive difference in the opportunity of species to cross may explain why some hybrids found in the wild have not yet been duplicated artificially e.g. Glyceria fluitans (L.) R.Br. x G. plicata (Fries) Fries (Borrill, 1975).

2.2 Embryo viability

Crosses where embryo death occurs between zygote formation and seed maturity have been called seed-incompatible by Valentine (1956), who has studied the phenomenon in some detail in Primula. Valentine (1961) used the strength of interspecific seed incompatibility as an indication of phylogenetic affinity in this genus, although Eaton (1973) later expressed doubts on the extent of its value for such purposes.

Hybrid seed inviability is a common phenomenon and Stebbins (1958) has classified the causes of postzygotic death as incompatibilities between a) the chromosomal material of one parent and the cytoplasm of the other, b) the chromosomal material of the two parents, and c) the hybrid embryo and the endosperm or maternal tissue. The distinction between inter-chromosomal and chromosomal-cytoplasmic incompatibility may be difficult to recognise in practice, but generally the latter is marked by reciprocal hybrid differences while the former is not (Stebbins, 1958). Stebbins (1958) considered interchromosomal incompatibility to be due either to general effects or to effects of specific genes and examples of both are known.

Brink and Cooper (1947) studied the embryo-endosperm relationship and found that the embryos of inviable hybrids appear to possess the potential for initiating growth, but are prevented from reaching adult size and character. Successful development of the embryo depends upon a continuous supply of nutrients from the endosperm. It has been found in many interspecific crosses that the collapse of the embryo is preceded by the disintegration of the endosperm (Dale, 1976).

The aseptic culture of embryos, developed in cereals mainly for plant breeding purposes, has often been used for providing an alternative nutrition supply to hybrid embryos which would otherwise abort or not germinate. The technique has also been used in the production of plants from monoploid barley caryopses which result from chromosome elimination in the interspecific hybrid Hordeum vulgare (female) x H. bulbosum (male) (Kasha & Kao, 1970). In the developing caryopsis the endosperm develops for two to five days and then disintegrates, but the embryo can be successfully grown by embryo culture (Konzak, Randolph & Jensen, 1951). Davies (1960) cultured embryos from interspecific hybrids of Hordeum twelve days after pollination. Hormone treatment of pollinated spikes from one or two days after crossing improves the hybrid caryopsis set in barley (Jensen, 1974) and also enhances the development of the caryopsis (Jensen, 1975).

2.3 Hybrid plant inviability and sterility

In some cases the hybrid seed germinates but the plant does not reach adulthood because its growth is inhibited. Stebbins (1958) gives the types of incompatibility mechanisms that could be responsible as either incompatibility between parental genetic material or that involving cytoplasmic and plastid differences. Chlorotic seedlings are commonly encountered in the offspring of artificial hybridisation.

F_1 hybrids may be perfectly viable in the absence of competition but do not survive in the wild. Several examples are known in which viable hybrid seed has been collected in the wild from the maternal parent and grown in cultivation, but the F_1 hybrid itself has not been found in the wild. In Poland, Skalińska et al. (1971) found heptaploid caryopses on plants of Festuca rubra L., although all the adult plants sampled were either hexaploid or octoploid. Hybrid caryopses of Poa annua L. x P. infirma Kunth have also been collected from wild plants (Tutin, 1975), but the F_1 again has not been found in the wild. Sometimes F_1 hybrids may reach maturity but produce few or no flowers.

In most cases of hybridisation, the hybrids are normal in their vegetative development, but show some kind of reduction in fertility. It has often been pointed out that hybrid sterility is not correlated with plant vigour. In many cases very strong-growing hybrids are completely sterile, and because seeds are not formed, sterile hybrids may possess a far longer flowering period and vegetative growth phase than their parents (Stace, 1975). Most grass hybrids are sterile but many in the genera Lolium and Bromus, for example, are not (Stace, 1975).

Dobzhansky (1937), followed by Thompson (1940) and Stebbins (1950), recognised two basic causes of hybrid sterility: genic and chromosomal. Genic sterility is caused by particular cytoplasmic or chromosomal genes which govern interspecific incompatibility and whose action is delayed until the reproductive phase of the F_1 . Hybrid failure may take effect before meiosis occurs, during it or after it. Chromosomal sterility is the failure of synapsis during the first meiotic prophase due to varying degrees of non-homology of the chromosomes of the two parental species and is an extremely common form of isolating mechanism in plants.

In some cases chromosome pairing appears complete but the pollen and ovaries are non-functional. This is usually explained by the

concept of cryptic structural hybridity (Stebbins, 1945), whereby minute inversions or translocations have occurred, followed by nearly identical re-inversions and re-translocations, so that small parts of the chromosomes are misplaced. Without upsetting the gross features of synapsis this is sufficient either to cause desynapsis due to lack of chiasmata or to cause inviability of the meiotic products through deficiencies and duplications resulting from the interchange of minute segments.

Later, Stebbins (1958), Solbrig (1968) and others used a different system, where haplontic or gametic and diplontic or zygotic sterility is recognised. Haplontic sterility results in the gametes of the hybrid being non-functional, while diplontic sterility effects either the somatic tissue of the F_1 or the diploid F_2 hybrid embryo.

2.4 Meiotic studies

Soon after the significance of the chromosomes in heredity was realised, chromosome homology became an important factor in genome analysis. Pairing observations in first meiotic metaphase are taken as an indication of homology and the bivalents observed are considered as representing chromosomes having at least some structural segments in common.

This method was extensively used by Kihara and his co-workers, who succeeded in establishing a general picture of the relationships within the genera Aegilops and Triticum (Kihara, 1954; Lilienfield, 1951). Since then many workers have studied chromosome pairing at meiosis in interspecific hybrids as an insight into species relationships.

De Wet and Harlan (1972) pointed out some shortcomings of genome analysis at the higher ploidy levels. Autopolyploids are usually assumed to be recognisable by multivalent chromosome configurations during meiosis, while the chromosomes of allopolyploids are assumed to synapse into bivalents. Most natural polyploids (segmental allopolyploids),

however, fall somewhere in between these two extremes in genome constitution. They combine more or less well differentiated genomes of different ecotypes of a species, or genomes of closely related species (Löve, 1964).

In hybrids with a ploidy level of triploid or above which form only bivalents and univalents at first metaphase, it is often not known which chromosomes are pairing together. Bivalents could represent pairing between the chromosome complements of the two different original parents (heterogenetic or homoeologous pairing) or between the chromosome complements of the same original parents (homogenetic or homologous pairing). Tetraploid Cynodon dactylon (L.) Pers. ($2n = 36$) crosses readily with several different diploid species. Chromosomes in these triploid hybrids consistently associated into nine bivalents and nine univalents during meiosis, and C. dactylon was assumed to be an allotetraploid. However, some tetraploid C. dactylon individuals behaved cytogenetically like true autotetraploids, and detailed cytological study of this genus has revealed a complex of species with very little genome differentiation between taxa (Harlan et al., 1970).

Evidence has accumulated from many sources to show that chromosome pairing at meiosis is under genetic control (Rees, 1961). Riley and Law (1965) have described various instances of regulation of chromosome pairing by genetic control. A most striking example of gene-controlled pairing was first demonstrated in hexaploid wheat. Riley and Chapman (1958) have presented conclusive evidence that a gene present in chromosome 5B of Triticum controls "diploidisation" of hexaploid wheat. Triticum aestivum is a segmental allohexaploid ($2n = 6x = 42$) with a genomic constitution (AA BB DD) derived from the genomes of three diploid species, probably T. monococcum (A), Aegilops speltoides (B) and A. squarrosa (D). Only bivalents are normally formed at meiosis. Sears

(1954) established a complete series of monosomics and nullisomics in wheat, and, if chromosome 5 of the B genome is missing, homoeologous pairing (i.e. pairing between A, B and D genomes, forming multivalents) resulted.

There is a strong indication of a similar control of pairing in hexaploid oats (Rajhathy, 1971; Rajhathy & Thomas, 1972, 1974), tetraploid cottons (Kimber, 1961) and tetraploid tobacco (Riley & Law, 1965). Jauhar (1975) proposed from several lines of evidence that Festuca arundinacea Schreber ($2n = 6x = 42$) is a segmental allopolyploid and has a chromosome whose presence is essential for regular bivalent formation. Jauhar (1975) also suggested that F. rubra ($2n = 6x = 42$) is a segmental allopolyploid and that because only 21 bivalents are found at meiosis this species may also have a gene controlling bivalent pairing at meiosis. Jauhar examined Lolium perenne ($2n = 14$) x F. rubra ($2n = 42$) intergeneric hybrids cytologically and found that 14 bivalents were formed in 30% of the cells. 14 bivalents in this hybrid could only have resulted from the seven chromosomes of the L. perenne parent pairing with a F. rubra genome, and the remaining seven bivalents from homoeologous pairing between the other two F. rubra genomes. Jauhar suggested that because the presumed regulator is in the hemizygous state in the hybrids, homoeologous pairing could occur, but no homoeologous pairing was observed in hexaploid F. rubra because the presumed regulator would be in double dose. It is now known that there are a number of other genes in wheat that affect pairing apart from those on chromosome 5B; these are minor suppressors and promoters of pairing (Sears, 1977).

From the preceding account observations of chromosome pairing at meiosis can be used as a guide to species relationships, but the genetic factors influencing pairing must also be taken into consideration.

CHAPTER 3

THE GENERA FESTUCA AND VULPIA3.1 Introduction

The present study is concerned with evolutionary relationships between the Festuca rubra aggregate, or F. rubra sens. ampliss. as defined by Hackel (1882), and the genus Vulpia. The F. rubra agg. comprises a group of perennial fine-leaved fescues, some of which are very important as amenity grasses in Britain and are also sown for grazing on hill pastures. Species in the genus Vulpia are mostly annual. Twelve species occur in Europe (Stace & Cotton, 1980) of which four grow in Britain; the rest are distributed mainly in the Mediterranean region. There are about nine other species of Vulpia in the rest of the world (West Asia, North and South America). The two genera are thought to be closely related as there are morphological similarities and natural intergeneric hybrids occur (Willis, 1975).

3.2 The genus Festucai. Taxonomy

The genus Festuca, within which 170 European species are recognised by Markgraf-Dannenbergh (1980), is one of the largest genera of the tribe Poeae and of the subfamily Pooideae of the Poaceae.

Linnaeus (1754) first described the genus Festuca in *Genera Plantarum*, and the species in 1753, in *Species Plantarum*. In 1882, Hackel reviewed the genus in *Monographia Festucarum Europaeae*, basing his classification on the differences in plant morphology and anatomy and especially on differences in amount and position of sclerenchyma in the leaves of both the culms and sterile shoots. Hackel divided the genus into the following five sections: Ovinae, Bovinae, Subbulbosae,

TABLE 1. Classification of the Testuca rubra L. aggregate by Hackel (1882), Howarth (1923, 1949) and Markgraf-Dannenberg (1980) and their chromosome numbers (from Markgraf-Dannenberg, 1980).

Hackel (1882)	Howarth (1922)	Howarth (1948)	Markgraf-Dannenberg (1880)	Chromosome number (2n)
<i>F. rubra</i> subsp. heterophylla (Lam.) Hackel subvar. typica Hackel subvar. leiophylla Hackel subvar. velupoides (Schur) Hackel subvar. puberula (Pari.) Hackel <i>F. plicata</i> Hackel (in <i>F. ovina</i> agg.) <i>F. rubra</i> subsp. violacea (Schleicher ex Gaudin) Hackel var. genuina Hackel subvar. typica Hackel subvar. aurata (Gaudin) Hackel subvar. subracemosa (Schur) Hackel subvar. aureo-flava (Schur) Hackel subvar. carnica Hackel subvar. flaccida (Schur) Hackel var. iberica Hackel subvar. typica Hackel subvar. subscabra Hackel var. nigricans Hackel <i>F. clementi</i> Boiss. (in <i>F. ovina</i> agg.) <i>F. rubra</i> subsp. violacea (continued) var. picta (Kit.) Hackel var. norica Hackel subsp. eu-rubra Hackel var. fallax (Thuill.) Hackel var. genuina Hackel subvar. vulgaris Hackel subvar. grandiflora Hackel subvar. glaucescens (Hegetschw. & Heer.) Hackel subvar. juncea Hackel subvar. barbata (Shrank) Hackel subvar. arenaria (Osbeck) Hackel subvar. dasyphylla Celakovsky subsp. pyrenaica (Reuter) Hackel subsp. eu-rubra (continued) var. trichophylla (Ducros ex Gaudin) Hackel subvar. typica Hackel subvar. uliginosa (Schur) Hackel var. oelandica Hackel subsp. dumetorum Hackel subsp. eu-rubra (continued) var. batheri (Timb.-Lagr.) Hackel var. planifolia Hackel subvar. typica Hackel subvar. baicalensis (Griseb.) Hackel subvar. viliflora Hackel var. rivularis (Boiss.) Hackel subsp. novadensis Hackel	<i>F. rubra</i> subsp. fallax (Thuill.) Howarth <i>F. rubra</i> subsp. genuina Hackel var. vulgaris Gaud. var. grandiflora Hackel var. tenuifolia Howarth var. glaucescens (Hegetschw. & Heer.) Richt. var. juncea (Hackel) Richt. var. arenaria (Osbeck) Fr. var. dumetorum (L.) Gaud. <i>F. juncifolia</i> St.-Amans <i>F. rubra</i> subsp. genuina var. planifolia Hackel	<i>F. heterophylla</i> Lam. <i>F. rubra</i> subsp. fallax (Thuill.) Howarth var. commutata Gaud. var. vulgaris Gaud. var. tenuifolia Howarth var. pruinosa (Hackel) Howarth var. glaucescens (Hegetschw. & Heer.) Richt. var. juncea (Hackel) Richt. <i>F. rubra</i> subsp. fallax (Thuill.) Hackel var. barbata (Hackel) Howarth <i>F. rubra</i> subsp. duriuscula var. arenaria (Osbeck) Fr. var. oraria (Dum.) Howarth var. dumetorum (L.) Gaud. <i>F. juncifolia</i> St.-Amans <i>F. rubra</i> subsp. duriuscula var. planifolia Hackel	<i>F. heterophylla</i> Lam. <i>F. plicata</i> Hackel <i>F. violacea</i> Schleicher ex Gaudin (subsp. violacea (subsp. macranthera (Hackel ex G. Beck) Markgr.-Dannemb. (subsp. handelii Markgr.-Dannemb. <i>F. nitida</i> Kit. ex Schultes subsp. nitida subsp. flaccida (Schur) Markgr.-Dannemb. (<i>F. iberica</i> (Hackel) Patzke (<i>F. puccinellii</i> Parl. (<i>F. clementi</i> Boiss. <i>F. picta</i> Kit. <i>F. baffinensis</i> Polunin <i>F. norica</i> (Hackel) K. Richter (not in <i>F. rubra</i> agg.) (<i>F. nigrescens</i> Lam. (subsp. nigrescens (subsp. microphylla (St.-Yves) Markgr.-Dannemb. <i>F. rubra</i> subsp. rubra <i>F. rubra</i> subsp. littoralis (G.F.W. Meyer) Auquier <i>F. rubra</i> subsp. pruinosa (Hackel) Piper <i>F. rubra</i> subsp. juncea (Hackel) Soó <i>F. rubra</i> subsp. arenaria (Osbeck) Syme <i>F. rubra</i> subsp. thessalica Markgr.-Dannemb. <i>F. rubra</i> subsp. asperifolia (St.-Yves) Markgr. Dannemb. <i>F. richardsonii</i> Hooker <i>F. pyrenaica</i> Reuter <i>F. trichophylla</i> (Ducros ex Gaudin) K. Richter <i>F. pseudotrachelophylla</i> Patzke <i>F. cyrenaica</i> (Litard. & St.-Yves) Markgr.-Dannemb. <i>F. oelandica</i> (Hackel) K. Richter <i>F. juncifolia</i> St.-Amans <i>F. diffusa</i> Dumort. <i>F. rivularis</i> Boiss. <i>F. rothmaleri</i> Litard. <i>F. novadensis</i> (Hackel) Markgr.-Dannemb. <i>F. cretacea</i> (Lavrenko) T. Proskorj	28 14 14 28 28, 42 42, 56 42 42 42 56 742 42 56 42, 56 14, 42 70

Variae, Scariosae and Montanae. F. rubra falls in section Ovinae. This section is separated from other sections by the following characters: basal leaf-sheaths not thickened, leaf-blades either all conduplicate or sometimes culm leaves flat, ligules short, truncate, often biauriculate; spikelets elliptical or oblong-elliptical; lemmas narrow with scarious margins; ovary obovate-oblong, glabrous or rarely moderately hispid at the tip, with an exactly terminal style; caryopsis ventrally deeply furrowed, with hilum almost as long; lemma and palea tightly adherent to the caryopsis.

Hackel differentiated between the species in section Ovinae primarily with respect to branching pattern, and made the two subsections 'Intravaginales' and 'Extravaginales vel Mixtae'. Both sorts of branches arise at the lower stem-nodes in the axils of the leaf-sheaths; where they form a very acute angle with the main stem and are retained within the leaf-sheath for some distance they are termed intravaginal, and where they diverge widely and break through the leaf-sheath at its base they are termed extravaginal.

Hackel recognised only two species, F. rubra sensu amplissimo and F. porcii Hackel in subsection 'Extravaginales vel Mixtae'. The former has entire leaf-sheaths and glabrous ligules, whereas the latter has leaf-sheaths split almost all the way to the base and has densely ciliate ligules. The nine species falling in subsection 'Intravaginales' are F. ovina L., F. hystrix Boiss., F. clementei Boiss., F. plicata Hackel, F. morisiana Parl., F. amethystina L., F. scaberrima Lange, non Steudel (= F. capillifolia Dufour), F. ampla Hackel and F. henriquezii Hackel.

Hackel (1882) thus placed the whole of the F. rubra aggregate into one species F. rubra, which he divided into six subspecies and numerous varieties and subvarieties (see Table 1).

Howarth (1923, 1948) applied Hackel's classification to the

British fescues and raised the rank of most of Hackel's taxa. Thus subsp. eu-rubra became F. rubra and subsp. dumetorum became F. juncifolia; subsp. eu-rubra var. genuina and var. fallax became subsp. duriuscula and subsp. fallax; and most of the subvarieties of var. genuina became varieties of subsp. duriuscula (see Table 1).

Various experimental taxonomists have since studied F. rubra agg. Jenkin (1955) attempted hybridisations within the F. rubra agg. Kjellqvist (1961, 1964) looked at leaf anatomy, cytology, ecology and the distribution of Scandinavian populations of F. rubra. Kerguelen (1975) tried to clarify the nomenclature of the F. rubra agg. Huon (1970) studied the ecology and cytology of F. rubra in S.W. France. Auquier (1974) studied the ecology and leaf anatomy in the F. rubra agg. from Belgium and has recently (Auquier, 1977) reported on their degree of inbreeding and outbreeding. At the present Auquier is studying floral rhythms and hybridisation between species of the F. rubra agg. (pers. comm., 1979).

In Flora Europaea, Markgraf-Dannenberg (1980) recognised 23 European species in the F. rubra agg. as defined by Hackel (see Table 1) and about 70 species in the F. ovina agg. In the present study the circumspection of F. rubra is that described by Markgraf-Dannenberg (1980) and it has the following characters which separate it from the 22 other species in the F. rubra agg: loosely or rarely densely caespitose with rhizomes often present; non-flowering shoots extravaginal or intravaginal; sheath usually decaying into fibres; leaves (0.5-) 0.65 - 1.5 mm wide, plicate, rounded or weakly carinate on back, setaceous to junciform with 5 to 9 veins, with or without bulliform cells; leaf-blades ribbed on the adaxial side, separate strands of sclerenchyma present subepidermally in the abaxial side at each rib; panicle more than 5 cm; lemma glabrous or hairy but not whitish; awn not more than half as long as lemma.

ii. Cytology and distribution

The basic chromosome number for the fescues as for most other grasses in the subfamily Pooideae is seven. Polyploidy is of common occurrence and can be as high as decaploid in some species belonging to the Ovinae and the Bovinae. Malik and Thomas (1966) estimated that 74% of the species in the genus Festuca are polyploids and they suggested that from the extent of polyploidy, the wide geographical distribution and the large number of sub-species, varieties and sub-varieties that the genus is phylogenetically very old.

Chromosome numbers reported for species in the F. rubra agg. show that ploidy levels in this group range from diploid to decaploid (Markgraf-Dannenberg, 1980) (see Table 1). Many cytotaxonomists have reported chromosome numbers for members of the F. rubra agg. but, because the taxonomy of this group is confused and the provenance of material collected from the wild is not always clear due to the widespread use of agricultural varieties, caution is needed when using the cytological data. Details of very few karyotypes of F. rubra have been published. Flovik (1938, 1940) reported some chromosomes with secondary constrictions and satellites and one plant examined had a single B chromosome. Malik and Thomas (1966) published ideograms of hexaploid and octoploid F. rubra showing secondary constrictions and satellites on ten pairs of chromosomes in the hexaploid. All the chromosomes were either metacentric or sub-metacentric. Konsarka (1974) has presented photographs of root tip squashes but no karyotypes were worked out.

Markgraf-Dannenberg (1980) recognised seven subspecies of F. rubra with chromosome numbers varying from diploid to decaploid, though the precise allocation of these ploidy levels has not been made in all cases (Table 1). Chromosome counts of wild plants of F. rubra in Britain, France and Belgium, have shown that both hexaploids ($2n = 42$)

and octoploids ($2n = 56$) occur (Stace & Cotton, 1974; Huon, 1970; Auquier & Rammeloo, 1973).

There are two other native British species in the F. rubra agg: F. juncifolia and F. nigrescens. F. juncifolia, a sand-dune species, is morphologically distinct from F. rubra in having longer upper (5.5 - 8.6 mm) and lower glumes (4.2 - 6.7 mm) and lemmas (6.5 - 8.9 mm) (Table 36), rigid, inrolled leaf-blades with well-developed and sometimes continuous abaxial sclerenchyma, extensively creeping rhizomes, only extravaginal branches and an octoploid ($2n = 56$) chromosome number (Huon, 1970; Auquier & Rammeloo, 1973; Stace & Cotton, 1974). F. nigrescens (F. rubra subsp. commutata or Chewings fescue) is a densely tufted hexaploid (Huon, 1970; Auquier & Rammeloo, 1973) without rhizomes, which grows in open grassland and on waste ground. F. heterophylla, a naturalised species, also occurs in Britain and differs from F. rubra in having only intravaginal branches, finer basal leaf-blades and a hairy tip to the ovary.

F. violacea, found in the Alps, Appenines and mountains of the Balkan peninsula, F. picta, a calcifuge growing in the East Alps, Carpathians and mountains of Bulgaria, and F. clementei, a calcifuge growing in the Sierra Nevada (S. Spain), are diploids and may therefore be of significance in evolutionary relationships. F. rivularis, a calcifuge growing in the mountains of Spain and S. France, is reported to have both diploid and hexaploid cytotypes (Markgraf-Dannenberg, 1980).

iii. Hybridisation between F. rubra and other species of Festuca

Natural hybrids have been reported between F. rubra and F. glauca Vill., F. heterophylla and F. ovina but none of these has been found in Britain (Borrill, 1975). Reports of hybrids of F. rubra with F. pratensis Hudson, F. gigantea (L.) Vill. and F. juncifolia have been made, but none of these has been substantiated.

Jenkin (1955) attempted a number of artificial hybridisations between F. rubra and other fescues and the following results were obtained: hybrid plants were produced from the cross F. rubra (female) x F. arundinacea (male), but all the plants failed to produce inflorescences; germinable caryopses were obtained when F. rubra and F. heterophylla were crossed in either direction, but only one seedling in ten reached maturity; mature F_1 plants were established from F. ovina (female) x F. rubra (male) but they were pollen sterile and Thomas (1948) reported that pairing between F. ovina and F. rubra was weak. Crosses were also attempted between F. rubra and F. pratensis and F. capillata Lam. (= F. tenuifolia Sibth.); both crosses produced caryopses but either these failed to germinate or the seedlings did not reach maturity. Auquier (pers. comm., 1979) has also obtained hybrids between F. rubra and F. heterophylla and these have flowered.

3.3 The genus Vulpia

i. Taxonomy

All species which are nowadays included in the genus Festuca are perennial. The annual species which are very similar to the perennial fescues have been segregated into various annual genera. The largest of these is Vulpia but others, particularly Micropyrum, Ctenopsis, Wangenheimia and Castellia, contain closely related species. The generic limits of these annual species have been altered on innumerable occasions. In extreme cases (Ascherson & Graebner, 1900 - 1901; Hackel, 1887), all the annual species were included in Festuca.

Cotton and Stace (1977) surveyed a wide range of macro-morphological, micromorphological and anatomical characters and delimited Vulpia with four sections: section Vulpia (eleven species), section Loretia (five species), section Monachne (three species) and section

TABLE 2 Species of *Vulpia* allocated to sections (taken from Cotton & Stace, 1977 and Stace, 1978) and their chromosome numbers (taken from Cotton & Stace, 1975).

	Chromosome number
<u><i>Vulpia</i> C.C. Gmel. section <i>Vulpia</i></u>	
<i>V. antofagastensis</i> Parodi	
<i>V. bromoides</i> (L.) S.F. Gray	14
<i>V. ciliata</i> Dumort.	28
<i>V. eriolepis</i> (Desv.) Blom	
<i>V. hirtiglumis</i> Boiss. & Hausskn.	42
<i>V. microstachys</i> (Nutt.) Benth.	42
<i>V. muralis</i> (Kunth) Nees	14
<i>V. myuros</i> (L.) C.C. Gmel.	42
<i>V. octoflora</i> (Walt.) Rydb.	14
<i>V. persica</i> (Boiss. & Buhse) Krecz. & Bobr.	42
<i>V. sciurea</i> (Nutt.) Henr.	42
<u><i>Vulpia</i> section <i>Monachne</i> Dumort.</u>	
<i>V. fasciculata</i> (Forsk.) Samp.	28
<i>V. fontquerana</i> Melderis & Stace	14
<i>V. membranacea</i> (L.) Dumort.	14
<u><i>Vulpia</i> section <i>Loretia</i> (Duval-Jouve) Boiss.</u>	
<i>V. alopecuros</i> (Schousboe) Dumort.	14
<i>V. geniculata</i> (L.) Link	14
<i>V. ligustica</i> (All.) Link	14
<i>V. litardiereana</i> (Maire) A. Camus	
<i>V. sicala</i> (C. Presl) Link	14
<u><i>Vulpia</i> section <i>Spirachne</i> (Hackel) Boiss.</u>	
<i>V. inops</i> (Del.) Hackel	14
<u><i>Vulpia</i> section <i>Nardurus</i> (Reichb.) Stace</u>	
<i>V. unilateralis</i> (L.) Stace	14

Spirachne (one species). Later (Stace, 1978), Nardurus maritimus (the type species of the genus Nardurus) was added to Vulpia as a fifth section (section Nardurus) (Table 2).

The genus Vulpia has been segregated from Festuca on a number of characters. Vulpia species are characteristically annuals with cleistogamous florets, one to two stamens, narrowly cylindrical caryopses, lemma gradually attenuated into awn at least as long as the lemma and a lower glume less than $\frac{3}{4}$ the length of the upper glume. None of these differences, however, is absolute. V. sicula and V. litardiereana are perennials; V. sicula, V. geniculata, V. ligustica and V. alopecuroides have chasmogamous florets; V. octoflora and V. bromoides have relatively broad caryopses; V. octoflora has rather abruptly narrowed and shortly awned lemmas and also has a lower glume $\frac{7}{8}$ length of the upper glume; all these characters are typical of Festuca sensu stricto (Cotton & Stace, 1977).

In the present study only the European species in sections Vulpia, Monachne and Loretia were studied. Species in the largest section (Vulpia) are all cleistogamous and are rather unsuitable for hybridisation experiments. Sections Spirachne and Nardurus are monotypic and were not used.

The following characters distinguish sections Vulpia, Monachne and Loretia (Cotton & Stace, 1977). Species in section Vulpia are cleistogamous, usually with a single, small (0.3 - 0.8 mm) anther, although occasionally two or three anthers are found. The ovary is glabrous. The lemma callus is a thickened horseshoe-shaped structure approximately 0.1 to 0.2 mm long. In V. ciliata there are one to three fertile florets and three to seven distal sterile florets. The lemmas of the latter exceed those of the former in length and width but have short awns. The rest of the species in this section have 1 to 2 (3) of

the distal florets reduced and often sterile. The pedicels are not dilated. The bundles of sclerenchyma above and below the veins in the leaves are poorly developed.

Section Monachne is a group of semi-cleistogamous annuals with three anthers, 0.5 to 2 mm long. The ovary is pubescent at the apex in V. fasciculata but glabrous in V. membranacea and V. fontquerana. The lemma callus in V. membranacea and V. fasciculata is long (0.5 - 0.8 mm) and pointed and in V. fontquerana it is 1 to 1.5 mm and sharply pointed. In all three species there is a congested group of two to six very small sterile florets. The pedicels are distally dilated. The bundles of sclerenchyma in the leaves are better developed than in section Vulpia.

Species in section Loretia have a chasmogamous flowering habit and relatively large anthers (2 to 5 mm). V. sicula and V. litardiareana are the only perennials within this section and in the genus. All species have pubescent ovaries and a small horseshoe-shaped callus except V. alopecuroides which has a glabrous ovary and a long (0.5 - 0.8 mm) pointed callus. The pedicels are distally dilated and the sclerenchyma bundles in the leaves are better developed than in section Monachne.

ii. Cytology

Cotton and Stace (1976) surveyed previous chromosome counts for species of Vulpia and added many of their own counts, and found that each species possesses a characteristic chromosome number (Table 2). A conspicuous feature of the chromosome numbers of the genus Vulpia is the occurrence of diploids, tetraploids and hexaploids in section Vulpia, but only diploids in the four other sections except for V. fasciculata (tetraploid) in section Monachne.

The karyotypes of the species studied by Cotton and Stace (1976) were found to consist of very similar chromosomes. All the chromosomes had submedian or median centromeres and many had an arm ratio approaching 1.7 which is the arbitrary point of distinction between m and sm chromosomes (Levan et al., 1965). Satellite chromosomes were not consistently recognisable.

iii. Distribution

Cotton and Stace (1976) mapped the distributions of all Old World Vulpia species.

Section Vulpia. V. myuros and V. ciliata are widespread throughout southern and central Europe, reaching as far north as the northern Caspian area in the east and England and Ireland in the west. In most of Europe they are the commonest species of Vulpia and both of them extend eastwards at least as far as north-western India. V. bromoides extends further north than any other species (to northern Scotland and southern Sweden) and is markedly more western in distribution than V. myuros and V. ciliata. V. muralis extends northwards to Hungary and eastwards to Cyprus. It has become well established in Southern America, often under the name V. australis and as such it is frequently introduced into northern Europe in wool. V. hirtiglumis and V. persica differ markedly from all the above in being western Asian species, the former extending from south-eastern Anatolia and Syria to central Iran, and the latter from Iraq and Armenia to Afghanistan and Russian Tien Shan. V. octoflora, V. microstachys, V. sciurea, V. antofagastensis and V. eriolepis are endemic to America. The first three species occur in North and the last two in South America.

Section Monachne. V. fontquerana is the rarest Vulpia species, having been collected only near the type locality in Pinus pinea forests at Algaida in south-western Spain. V. membranacea extends as far east as the western edge of Libya and south-eastern France. It was formerly known as far north as Belgium and still occurs on the coastal dunes of Pas de Calais in northern France. V. fasciculata has a typical Mediterranean coastal distribution, stretching eastwards to Israel and northwards along the Atlantic coast to Britain, where it is predominantly a south-western species. It is conspicuously less common than V. membranacea in the Iberian Peninsula and in France is replaced by the latter species in the extreme north-west and in inland localities.

Section Loretia. The five species in section Loretia are all western or central Mediterranean in distribution. V. alopecuroides is confined to the Iberian Peninsula and adjacent parts of Morocco and Algeria, whereas V. geniculata extends further east to southern France, Corsica, Sardinia and Tunisia. V. ligustica extends almost as far west as V. alopecuroides and V. geniculata in North Africa but is absent from the Iberian Peninsula and extends eastwards to eastern Libya and Crete. V. sicula occurs from Sicily westwards to Morocco and northwards to south-eastern France, but is absent from the Iberian Peninsula. V. litardiereana is confined to high altitudes of the Grand Atlas of Morocco.

iv. Evolutionary relationships within the genus Vulpia.

Stace and Cotton (1976) suggested that species in sections Vulpia and Monachne are evolutionarily more advanced than those in section Loretia since the former consist of annuals with short anthers and wholly or partially cleistogamous flowers, whereas species of section Loretia have larger anthers, chasmogamous flowers and two species of section

Loretia are perennial. Auquier and Stace (1980) have reported abnormal flowering behaviour in some specimens of V. myuros, V. bromoides and V. ciliata, with 1 to 3 micranthous or 1 to 3 macranthous (with anthers (0.8) 1.0 - 1.5 (1.8) mm long) stamens or a mixture of both sorts of stamens in one floret. Similar observations have also been reported for non-European species from section Vulpia. Auquier and Stace (1980) also reported that species in section Vulpia sometimes have inflorescences with some chasmagamous florets and interpreted these observations as a reversion of these species to ancestral characters. These observations support the earlier view that section Vulpia is more advanced than section Loretia and has evolved from the Loretia-type characters. Section Monachne is a well-defined taxon somewhat in between sections Vulpia and Loretia.

All species of section Loretia are diploid and are confined to west and central Mediterranean regions. Section Monachne contains one tetraploid and two diploid species, one of the diploids, V. fontquerana, is the most restricted species of all. Polyploids occur in section Vulpia and are much more widely distributed than the diploids. The successful spread of polyploids in section Vulpia again suggests the advanced specialisation of these species as contrasted with the totally diploid and more restricted section Loretia with species with the perennial habit, which is presumably a primitive feature derived from the totally perennial genus Festuca.

Cotton and Stace (1976) suggested that the genus Vulpia arose in one area which is now the western Mediterranean, and then spread northwards and eastwards as well as westwards to America. Thus western Asia and America should be considered secondary areas of diversity.

No natural interspecific Vulpia hybrids are known. However,

Cotton (1974) obtained one F_1 hybrid plant using V. fasciculata ($2n = 28$) as the female and V. geniculata ($2n = 14$) as the male parent. 115 pollinations were made and 46 caryopses were obtained; many of these were small and wrinkled and only one caryopsis germinated. In general appearance, length and branching of the inflorescence, the hybrid was intermediate between its two parents. In spikelet length, lemma length and upper glume length it was much closer to V. fasciculata. It was completely chasmogamous, triploid ($2n = 21$) and was completely sterile. At pollen mother cell meiosis seven bivalents were formed with one or two ring bivalents.

3.4 Intergeneric hybrids between F. rubra agg. and Vulpia.

Species of Vulpia share many characteristics with F. rubra agg. In particular, section Loretia has its perennial habit (in two species), chasmogamous florets and large anthers in common with F. rubra. The occurrence of natural intergeneric hybrids between Vulpia and F. rubra agg. suggests that there might also be a close evolutionary relationship between the two genera.

Natural intergeneric hybrids ($\times$ Festulpia Melderis ex Stace & Cotton) have been found between F. rubra and F. juncifolia with V. fasciculata and between F. rubra with V. myuros and V. bromoides. The cleistogamous and semi-cleistogamous nature of these species of Vulpia would apparently prevent any possibility of cross-pollination. However, Stace and Auquier (1980) reported abnormal flowering behaviour in species of Vulpia. In the typically cleistogamous section Vulpia, chasmogamous florets with micranthous or macranthous stamens have been observed in both V. bromoides and V. myuros. Also, Stace and Auquier (1980) reported considerable variation in the degree of chasmogamy in section Monachne.

The hybrids between V. fasciculata ($2n = 28$) and F. rubra ($2n = 42$) were first reported by Melderis (1955) and were collected from Southport, S. Lancs, and from Sandwich, E. Kent. M.D. Hooper studied some intergeneric hybrids cytologically and counted $2n = 35$ in two specimens from W. Sussex and $2n = 35, 42$ in specimens from E. Kent (Stace & Cotton, 1974). Stace and Cotton (1974) collected pentaploid hybrids ($2n = 35$) identifiable with F. rubra x V. fasciculata (x Festulpia hubbardii Stace & Cotton) from seven British localities from Kent to Glamorgan, but a hexaploid hybrid ($2n = 42$), identifiable with F. juncifolia x V. fasciculata (x Festulpia melderisii Stace & Cotton) was only found at Littlehampton, W. Sussex.

Morphologically, the F. rubra agg. x V. fasciculata hybrids are easily recognised as they are intermediate in floral morphology between the parents. The most reliable character for distinguishing them is the glume length ratio, which is less than 1:10 in V. fasciculata, approximately 1:2 in the hybrids and about 2:3 to 4:5 in the F. rubra agg. Stace and Cotton (1974) found that lemma pubescence of the hybrid varies considerably depending on the F. rubra parent and so this was not a reliable character to distinguish between pentaploid and hexaploid hybrids as had been previously suggested. However, in the hybrids examined by Stace and Cotton (1974), x Festulpia hubbardii had an upper glume length 5.0 to 8.0 mm and a lemma length 6.0 to 9.5 mm and x Festulpia melderisii 8.0 to 11.5 mm and 9.5 to 11.5 mm respectively. Therefore, the two hybrids can be distinguished morphologically and this can be verified by chromosome counts.

In growth habit the hybrids were more or less rhizomatous perennials, although, because of the variable nature of the F. rubra agg., the range of variation of the hybrids was considerable. Branching in F. juncifolia was extravaginal only, that in F. rubra both intravaginal and extravaginal and that in V. fasciculata only intravaginal. The

hybrids had both intravaginal and extravaginal branches but in the material observed by Stace and Cotton (1974) the latter were far more common and in the hexaploid from Littlehampton were the only sort. Pentaploid hybrids have been found in a range of habitats on sand-dunes, from sparse grassland to open, mobile dunes (Stace & Cotton, 1974).

When flowering, the anthers and the stigmas of the hybrids were well exerted (much more so than in V. fasciculata). Anthers were intermediate in size (1.5 - 2.0 mm long) between those of their parents, but stainable pollen was often 0% and was not observed over 3% (Stace & Cotton, 1974). Cotton had no success in germinating caryopses although Willis (1967) had recorded less than 1% fertility. In studies of pollen mother cell meiosis in pentaploid hybrids 10 to 14 bivalents plus 7 to 15 univalents were observed (Stace & Cotton, 1974).

The natural hybrid F. rubra x V. bromoides was found at Littlehampton, W. Sussex in 1961 (Melderis, 1965) and at Shingle Street, E. Suffolk in 1961 (Trist, 1971). The latter was found again by Trist in 1976. The plant is similar in general appearance to F. rubra, but the shape of the lemma, the length of the lemma awn and the glume ratio indicate the influence of V. bromoides. The hybrid is sterile (Willis, 1967; 1975).

The F. rubra x V. myuros hybrid was found at Arthog, Merioneth (Benoit, 1958), at Stockport, Cheshire by R. Cotton and C.A. Stace in 1974 and at Snettisham, Norfolk by R.P. Libbey. The hybrid is a densely caespitose perennial, without creeping rhizomes. It is intermediate between the parents in the exertion of the panicle, the number of nodes of the panicle, the width of the lemma and the length of the lemma awn. The hybrid is sterile (Willis, 1975). Cytological investigations have not been made on these latter two natural inter-generic hybrids.

Cotton (1974) attempted artificial hybridisation between F. rubra and V. alopecuroides, V. geniculata, V. fasciculata, V. sicula. The only successful cross was V. fasciculata (female) x F. rubra (male) in which 60 F₁ hybrids were obtained from 63 caryopses following 139 pollinations. 75 reciprocal pollinations were made but only one caryopsis was produced and this did not germinate. The hybrids were perennials and the seedlings easily distinguished from selfed V. fasciculata seedlings by their deep, red, lower leaf-sheath in contrast to the glabrous pale green leaf-sheath of V. fasciculata (Stace & Cotton, 1974). The hybrids were studied cytologically and were found to be pentaploid; at pollen mother cell meiosis about seven bivalents were formed (Cotton, 1974).

3.5 Aims of the present study

The aim of this study was to investigate the relationships and ancestry of Vulpia and F. rubra agg. ^{In Vulpia,} within section Vulpia (eleven species) there are diploids, tetraploids and hexaploids; within section Monachne (three species) there is one tetraploid and two diploids; and in section Loretia all five species are diploid. The relationships between the diploid species and between the diploid and polyploid species were investigated by interspecific hybridisation and consequent meiotic analysis and fertility of the F₁ hybrids. Morphological characters of the F₁ hybrids were also examined. The possible autotetraploid origin of V. fasciculata from one of the present day diploid species of Vulpia was tested by chromosome doubling of the diploid species. Giemsa banding of the chromosome of different species of Vulpia was attempted to see whether the otherwise very uniform karyotypes were distinguished by different banding patterns.

To examine the relationship between the genus Vulpia and F. rubra agg., specimens of naturally occurring hybrids were examined

cytologically to test the homology of the parental chromosomes; the fertility of the hybrids was also examined. Artificial hybridisation between those species that hybridise naturally was attempted, to reproduce the event which occurred in the wild and to compare the natural and artificial hybrids.

Artificial hybridisation between Vulpia species in section Loretia and F. rubra agg. was also attempted, as these species (and especially the perennial species) share many characters, and the perennial species of Loretia are probably the species most closely related to F. rubra agg.

Species in F. rubra agg. vary in ploidy level from diploid to decaploid. Hybrids between diploids are much easier to analyse cytologically than those between higher ploidy levels, and therefore plants of some diploids of the F. rubra agg. were obtained to hybridise both with diploid species of Vulpia and also other species of F. rubra agg.

Embryo culture of hybrid caryopses was employed to improve germination in caryopses which had an abnormal endosperm and also to remove the need of a post-harvest dormancy period so that the hybrids could be grown to maturity more quickly.

CHAPTER 4

MATERIALS AND METHODS

4.1 Source of material

The plant material used in this study has been accumulated by personal collection and by seed exchange over many years. The original localities of origin, together with the chromosome number where known, are listed in the Appendix.

4.2 Mitotic preparations

Chromosome numbers for species of Festuca and Vulpia and interspecific and intergeneric hybrids were determined by examination of mitosis in the root tips. Root tips were collected from either the growing plants or from germinating caryopses. Caryopses were grown in a covered petri dish on three layers of filter paper, moistened with water. The petri dish was sealed with Nescofilm to retain the moisture, and kept at a temperature of 18 - 20°C.

Cotton and Stace (1976) tried several pretreatment agents with Vulpia root tips but found a saturated solution of Gammexane (γ -hexachlorocyclohexane) to produce the most satisfactory results and therefore this pretreatment was used in the present study.

The following schedule was used:

- 1) Preheat the root tips in a saturated solution of gammexane at room temperature for three hours.
- 2) Wash free of gammexane in tap water.
- 3) Fix in 3:1 absolute alcohol: glacial acetic acid overnight in a refrigerator.
- 4) Hydrolyse in 1N hydrochloric acid for 20 minutes with Vulpia and for 30 minutes for x Festulopia and Festuca, in both cases at 60°C.

- 5) Rinse in tap water.
- 6) Excise root tips and stain in a 1:1 mixture of 1% acetocarmine (with ferric acetate) and 1% acetic-orcein.
- 7) Tap coverslip to separate cells and squash to flatten them.
- 8) Warm slide over flame to intensify stain.
- 9) Seal with rubber solution and make observations.

4.3 Meiotic preparations

Pollen mother cell meiosis in both parental species and hybrids was studied using the following procedure:

- 1) Fix young inflorescences in 3:1 absolute alcohol:glacial acetic acid and store in refrigerator.
- 2) Dissect off anthers on a black tile.
- 3) Stain anthers in a 1:1 mixture of 1% acetocarmine (with ferric acetate, and 1% acetic-orcein on a slide.
- 4) Tease out anthers in the stain and tap out the cells under a coverslip.
- 5) Warm the slide over a flame to intensify stain and squash to flatten the cells.
- 6) Seal the coverslip with rubber solution and make observations.

4.4 Pollen "fertility"

Pollen fertility of the hybrids was determined by staining the pollen grains in Muntzings aceto-carmine. Only those pollen grains with full, deeply stained cytoplasm were counted as fertile. There can be no doubt that empty pollen grains are sterile, but this method does not differentiate viable from inviable filled grains. The viability of filled pollen grains can be tested by their germination on agar.

4.5 Self-fertility experiments

The degree of self-fertility was determined by bagging the inflorescence the day before anthesis and tapping the bag every day until anthesis had finished. The inflorescences were harvested when ripe and the number of caryopses were expressed as a percentage of the total number of fertile florets (i.e. the florets on a spikelet with both stigmas and stamens).

4.6 Hybridisation experiments

Artificial hybridisation was attempted between eight different species of Vulpia, and between nine species of Vulpia and various taxa within F. rubra agg.

The following species of Vulpia were used in the hybridisation programme: V. alopecuroides, V. bromoides, V. fasciculata, V. fontquerana, V. geniculata, V. ligustica, V. membranacea, V. myuros and V. sicula. Several sources of known wild origin of each parental species were used to provide greater genetic variation and therefore a greater chance of success. In the case of V. fontquerana, however, only one seed source (originally the type collection) was available.

Vulpia caryopses were sown at three weekly intervals from mid-November to March in both Leicester and Aberystwyth. The seedlings were potted up individually and placed in a heated greenhouse (10°C) in Leicester and in an unheated greenhouse in Aberystwyth. The period from sowing to flowering for Vulpias was approximately three months. Some of the fescues were grown in experimental plots outdoors and others were tillered and grown in pots in an unheated greenhouse.

The crossing experiments took place from the end of April to the end of June in both 1977 and 1978. The degree of self-fertility varied from complete self-fertility in the cleistogamous and semi-cleistogamous species to high, though mostly incomplete self-sterility

in the chasmogamous species. Therefore all species used as the female parent in crosses were emasculated. Inflorescences to be used as the male parent were bagged with transparent envelopes made of thin, glazed paper on the day before expected anthesis, the anthers appearing either yellow or purple and swollen inside the closed florets. Dehiscence of the stamens followed very soon after their exsertion; there was no prolonged period when undehiscent anthers were protruding from the open florets. On tapping the flowering inflorescence ripe pollen accumulated in the bag. The pollen was always used within one or two hours of dehiscence so that it was fresh.

A number of spikelets were cut off the inflorescences to be used as female parents, so that a line of spikelets was left down one side of the main axis of the panicle. As many florets as possible were emasculated on these spikelets, those not emasculated being cut off. Emasculation was always carried out under a binocular microscope.

The lemma and palea were left intact but gently separated using fine forceps and a needle. The anthers could then be removed, care being taken not to burst them. After emasculation the lemma and palea were pressed together to avoid drying out of the stigmatic surface. The stigmas of the emasculated florets took two to four days to ripen depending on the heat and light conditions in the greenhouse. The female inflorescence was bagged immediately after emasculation. The bag was removed from the female inflorescence just before pollination, a soft paint brush was used to transfer the pollen to the ripe stigmas and the bag was replaced immediately afterwards. Pollination was also carried out under a microscope. If pollen was available the pollination process was repeated for a few days as long as the stigmas appeared receptive. Brushes used in pollination were sterilised in absolute alcohol after each cross and the microscope was also wiped with absolute alcohol.

Hybrid caryopses were harvested four to five weeks after pollination and the general appearance of the caryopses was examined.

4.7 Embryo culture

Seedlings from the hybrid caryopses were required as soon as possible so that mitotic and meiotic investigations could proceed. Therefore to break the post-harvest dormancy period (Watkinson, 1978) a cold treatment was applied to the imbibed caryopses.

The hybrid caryopses were sterilised in a 10% solution of Domestos, sown on moist filter paper in petri dishes and put in an incubator at 20°C. After 24 hours the imbibed caryopses were placed in a dark incubator at 5°C for seven days.

To extract and culture the embryos a procedure was used which was known to work with x Festulolium hybrids at W.P.B.S., Aberystwyth (pers. comm. M.W. Humphreys) and had been used at Leicester for barley embryos (pers. comm. K.J. Webb). The procedure used at Aberystwyth differs from the one used in this work, in that it uses embryos 9 - 12 days after they have been fertilised. In the present work, immature embryos were not employed as it was not until the mature caryopses had been examined that the deficient endosperm was revealed.

Procedure

The embryo culture medium used was Gambourg and Miller's B₅ medium minus nicotinic acid, as used by Kasha and Kao (1970) for barley embryos. Table 3 lists the ingredients. The stock solutions A (macro-nutrients), B (micronutrients), C (vitamins), D and E (iron source) were made up separately and stored in a refrigerator. One litre of A was made at x 10 concentration; 250 ml of B were made at x 1,000 concentration; 200 ml of C were made at x 100 concentration; 200 ml of D were made at x 100 concentration; and 200 ml of E were made at x 200 concentration.

TABLE 3 Gambourg & Miller's B₅ medium minus nicotinic acid

A. $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	150.0 mg/l	B. $\text{MnSO}_4 \cdot \text{H}_2\text{O}$	10.0 mg/l
KNO_3	2.5 mg/l	H_3BO_3	3.0 mg/l
$(\text{NH}_4)_2\text{SO}_4$	134.0 mg/l	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	3.0 mg/l
$\text{CaCl}_2 \cdot \text{H}_2\text{O}$	150.0 mg/l	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	250.0 $\mu\text{g/l}$
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	250.0 mg/l	CuSO_4	25.0 $\mu\text{g/l}$
		$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	25.0 $\mu\text{g/l}$
		KI	750.0 $\mu\text{g/l}$
C. Nicotinic acid	1.0 mg	D. Fe EDTA	6.9 mg/l
Thiamine.HCl	10.0 mg	Na_2 EDTA	9.3 mg/l
Pyridoxine.HCl	1.0 mg	E. Na_2 EDTA	18.6 mg/l
m-Inositol	100.0 mg		
Sucrose	20.0 g		
Agar	6.0 g		

Then 100 ml of A, 1 ml of B, 10 ml of C, 10 ml of D and 5 ml of E were put in a volumetric flask, the sucrose was added and the volume was made up to one litre with distilled water. The pH of the medium was adjusted to 5.8. The agar was added to the solution and the medium was heated in a steamer. The hot nutrient agar was transferred to glass vials using an automatic 10 ml pipette. Metal caps were put on the vials. Sterilisation was accomplished by autoclaving at 121°C at one atmosphere. After sterilisation the culture vials were placed at an angle of 45 degrees until the agar set.

The removal of the embryos from the caryopses was carried out under sterile conditions in a Laminar flow cabinet. The caryopses were disinfected in a 10% solution of Domestos for 5 - 7 minutes and rinsed in three changes of sterile water. Dissection was carried out under a binocular microscope. The lemma and palea were removed from the caryopsis and a slit was made with needles along the hilum towards the embryo. The heart-shaped embryo could be seen quite clearly, removed from the endosperm and placed on the surface of the agar in the vial.

It was important that no trace of ethanol remained on the needles used for dissection and that they were allowed to cool to room temperature after flame sterilisation. Care had to be taken with the microscope light source so that the heat emitted did not dry the embryo while it was dissected.

The vials were transferred to a darkened incubator at 25°C . The vials containing embryos which had germinated were placed in an incubator at 25°C under continuous light. After three weeks, when the seedlings had two or three leaves and several roots, they were very carefully removed from the agar, the roots were washed with sterile water and the seedlings were potted up individually in sterilised soil. An inverted glass tube was placed over each seedling to prevent desiccation

during the first few days. After 10 days the seedlings were transferred to a mist unit in a greenhouse (temperature 15°C) and when a few more leaves had grown they were transferred to a cooler part of the greenhouse (10°C) and were grown under lights.

For every litre of medium used 100 embryos could be grown. The whole experiment was repeated six times: four times one litre of medium was used and twice two litres of medium was used.

The embryo culture experiments were initiated about a month after the hybrid caryopses were harvested.

4.8 Colchicining of diploid species of *Vulpia*.

The method followed is that used at the W.P.B.S., Aberystwyth (pers. comm. G. Morgan). Caryopses were germinated on moist filter paper until about 3 cm of shoot had grown. The caryopses were then put into 0.2% colchicine solution for five hours. Then the caryopses were washed for two hours and were put into trays of soil and covered with an inverted filter funnel to prevent desiccation. When the seedlings had grown to a sufficient size, the plants were repotted and root tips were taken for chromosome counting.

Inflorescences were collected for meiotic study when the plants flowered, and any seed was harvested. The seed was grown on and, when the plants were mature enough, root tips were again taken for chromosome counting.

Chromosome number doubling was attempted for the following diploid *Vulpias*: *V. alopecuroides*, *V. geniculata*, *V. ligustica* and *V. membranacea* (three genotypes).

4.9 Giemsa banding

The following method of Giemsa banding of Lolium temulentum and some fescues, used by H.M. Thomas at the W.P.B.S., Aberystwyth, was adapted for the present study.

- 1) Pretreat root tips in distilled water at 1°C for 16 hours.
- 2) Fix root tips in ethanol:acetic acid (3:1) for 1 hour.
- 3) Soften root tips in 0.2N HCl at 60°C for 3 minutes.
4. Make squash preparations in 45% acetic acid on albuminised slides.
5. Remove coverslips by freezing the preparations.
6. Leave slides to air-dry overnight at room temperature.
7. Immerse slides in 45% acetic acid at 60°C for 20 minutes.
8. Rinse slides in tap water and place in a saturated solution of barium hydroxide for 5 minutes at room temperature.
9. Rinse slides thoroughly in tap water for 5 minutes.
10. Immerse slides in 2 x SSC (saline sodium citrate) for 5 minutes at room temperature before incubation in 2 x SSC at 60°C for 1 hour.
11. Rinse slides in water, air-dry and stain in 2% Giemsa solution for 10 minutes (Gurr's improved R66 in Sorensen buffer pH 5.9).
12. Rinse and air-dry slides and mount in Canada balsam.

CHAPTER 5

RESULTS

5.1 Breeding behaviour

The flowering behaviour of the species used in this study is listed below.

<u>Species</u>	<u>Flowering behaviour</u>
V. bromoides	cleistogamous
V. myuros	cleistogamous
V. fasciculata	semi-cleistogamous
V. fontquerana	semi-cleistogamous
V. membranacea	semi-cleistogamous
V. alopecuros	chasmogamous
V. geniculata	chasmogamous
V. ligustica	chasmogamous
V. sicula	chasmogamous
F. juncifolia	chasmogamous
F. rubra	chasmogamous

All the cleistogamous and semi-cleistogamous species are 100% self-fertile. Table 4 shows the self-fertility results for the four chasmogamous species of Vulpia and for F. rubra. The seed accession numbers used for each species are shown and the locality for each accession number is listed in the Appendix.

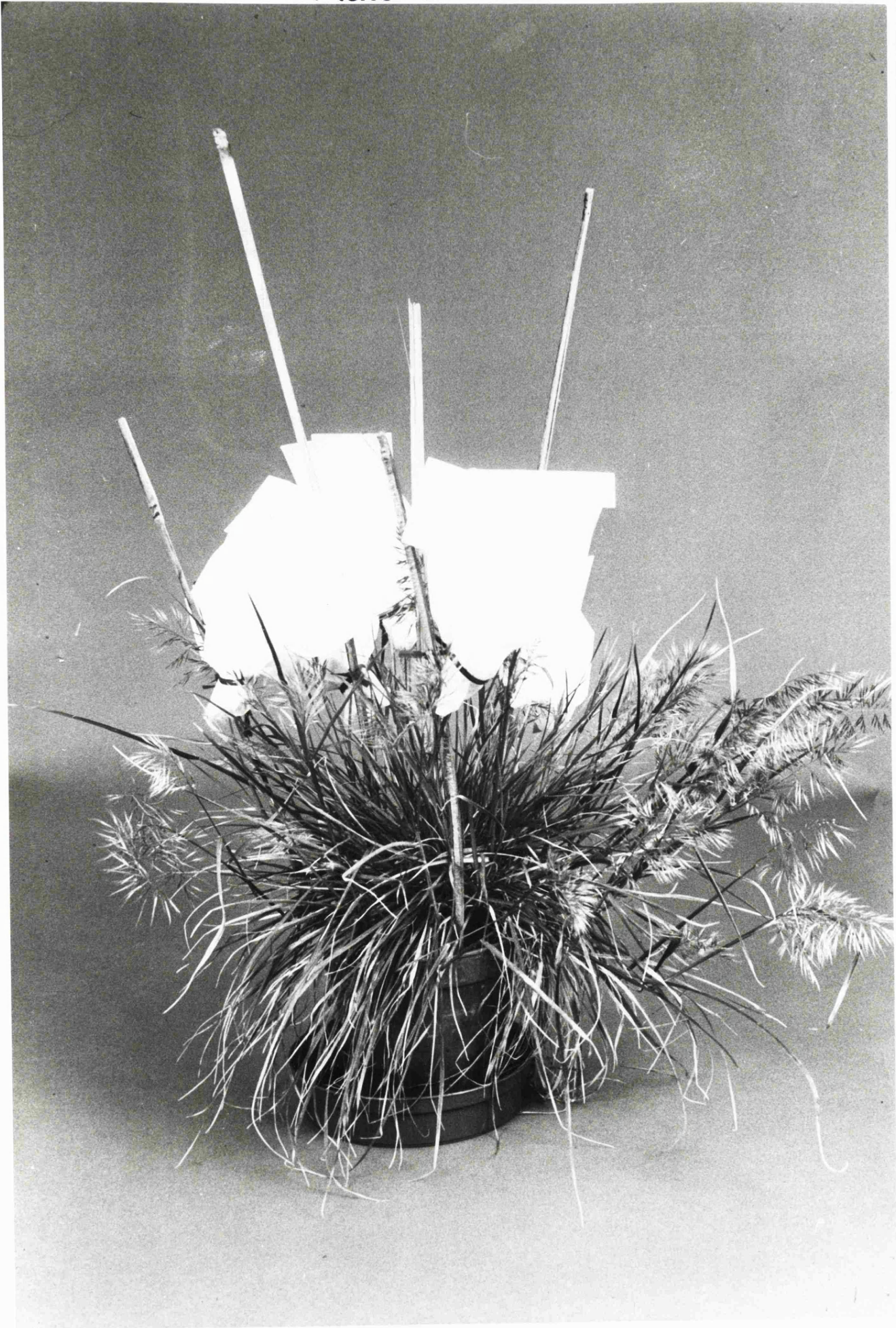
5.2 Artificial hybridisationi. Hybridisation experiments

Hybridisation experiments were carried out from the end of April to the end of June in 1977 and 1978. In 1978, crosses were tried which either failed or had not been attempted in the previous year. In the spring and early summer of 1978, the damp, cool conditions, especially in Aberystwyth, encouraged the spread of mould on those inflorescences bagged for crossing, and the production of hybrid caryopses was probably adversely affected by these conditions. Plate 1 shows emasculated inflorescences bagged for crossing.

TABLE 4 Self-fertility results

Species	Accession number	Number of plants	Range of % seed set when self-fertilised
V. alopecuroides	V79	5	0%
	V240	2	53 - 62%
V. geniculata	V356	4	0 - 5%
V. ligustica	V254	6	0%
V. sicula	V365	12	0 - 8.7%
	V479	2	0%
F. rubra	F35	3	0 - 1%
	F44	1	0%
	F45	3	1 - 2%
	F68	1	0%
	F70	1	3%
	F78	1	0%

Plate 1 Emasculated inflorescences of V. ligustica bagged for crossing.



V. geniculata and V. ligustica were the earliest Vulpia species to flower, V. myuros and V. fasciculata were the latest and the others were in between. A range of sowing dates for each species allowed different species to overlap in flowering times so that hybridisation could be effected. The flowering period of all species of Vulpia could be prolonged by removing the young inflorescences.

F. rubra and F. juncifolia flowered from the end of May to the beginning of July. Those plants growing in pots produced only a few inflorescences and therefore the number of crosses attempted with a fescue as the female parent was limited. Mature plants of F. rivularis, F. violacea and F. picta did not flower in two years.

Anthesis in the fescues occurred between 11 a.m. and 3.30 p.m. In Vulpia anthesis seemed to depend on the amount of sunshine and occurred on a sunny day any time between 12 a.m. and 4 p.m. Occasionally, anthesis in Vulpia was artificially induced by placing the plants under mercury vapour lamps.

The hybridisation results for 1977 and 1978 have been combined in tables 5 to 7. Crosses using different genotypes did not obviously differ from each other and therefore the results have been expressed collectively under the species. In the tables and in the following account the first mentioned species in a cross is the female parent. The three tables 5, 6 and 7, representing interspecific Vulpia crosses, intergeneric x Festulpia crosses and interspecific Festuca crosses respectively, are subdivided according to the ploidy levels of the parental species.

The variation in number of florets emasculated and pollinated in the different crosses depended upon the number of plants of the two species flowering at the same time, and on the ease of emasculation and pollen collection. Within Vulpia section Loretia, all crosses and

TABLE 5a Hybridisation results for *Vulpia* interspecific crosses. Diploid-diploid combinations.

Female	Male	No. of florets pollinated	No. of caryopses formed	
			Total	Selfs
1. <i>V. alopecuroides</i> (V79,V240,V33)	x <i>V. geniculata</i> (V82,V356)	435	0	
2. <i>V. alopecuroides</i> (V79,V240,V30)	x <i>V. ligustica</i> (V254,V255,V257)	465	0	
3. <i>V. alopecuroides</i> (V79)	x <i>V. membranacea</i> (V5,V65)	127	3	0
4. <i>V. alopecuroides</i> (V240,V30,V33)	x <i>V. sicula</i> (V365)	266	0	
5. <i>V. bromoides</i> (V110)	x <i>V. sicula</i> (V365)	12	0	
6. <i>V. fontquerana</i> (V404)	x <i>V. alopecuroides</i> (V30,V33)	95	13	1
7. <i>V. fontquerana</i> (V404)	x <i>V. geniculata</i> (V82,V356)	16	0	
8. <i>V. fontquerana</i> (V404)	x <i>V. ligustica</i> (V254,V257)	106	4	0
9. <i>V. fontquerana</i> (V404)	x <i>V. sicula</i> (V365)	37	0	
10. <i>V. geniculata</i> (V82,V356)	x <i>V. alopecuroides</i> (V30,V79)	111	0	
11. <i>V. geniculata</i> (V82)	x <i>V. ligustica</i> (V254,V255)	200	0	
12. <i>V. geniculata</i> (V82)	x <i>V. membranacea</i> (V364)	72	0	
13. <i>V. geniculata</i> (V82,V365)	x <i>V. sicula</i> (V365,V379)	295	2	0
14. <i>V. ligustica</i> (V254,V255)	x <i>V. alopecuroides</i> (V33,V30)	78	0	
15. <i>V. ligustica</i> (V254,V255)	x <i>V. geniculata</i> (V82,V356)	212	141	0
16. <i>V. ligustica</i> (V254)	x <i>V. membranacea</i> (V364)	56	0	
17. <i>V. ligustica</i> (V254)	x <i>V. sicula</i> (V365)	216	1	0
18. <i>V. membranacea</i> (V36,V65,V364)	x <i>V. alopecuroides</i> (V79,V240)	197	43	0
19. <i>V. membranacea</i> (V36,V65,V5, V364)	x <i>V. geniculata</i> (V82,V356)	208	34	0
20. <i>V. membranacea</i> (V36,V5, V136,V181)	x <i>V. ligustica</i> (V255,V254)	242	46	3
21. <i>V. membranacea</i> (V5,V36, V364,V181)	x <i>V. sicula</i> (V365,V379)	297	13	1
22. <i>V. sicula</i> (V365)	x <i>V. alopecuroides</i> (V33,V30)	419	0	
23. <i>V. sicula</i> (V365,V379)	x <i>V. geniculata</i> (V82,V356)	548	140	0
24. <i>V. sicula</i> (V365,V379)	x <i>V. ligustica</i> (V254,V257)	287	0	
25. <i>V. sicula</i> (V365)	x <i>V. membranacea</i> (V364)	84	0	

All hybrids counted proved to be diploid.

TABLE 5b Hybridisation results for *Vulpia* interspecific crosses. Diploid-tetraploid combinations

Female	Male	No. of florets pollinated	No. of caryopses formed	
			Total	Selfs
1. <i>V. alopecuroides</i> (V30,V240)	x <i>V. fasciculata</i> (V263,V294)	109	0	
2. <i>V. fasciculata</i> (V260,V263)	x <i>V. alopecuroides</i> (V79)	138	2	1
3. <i>V. fasciculata</i> (V260,V263, V 294)	x <i>V. geniculata</i> (V82, V356)	116	15	0
4. <i>V. fasciculata</i> (V260,V263)	x <i>V. ligustica</i> (V254, V255)	100	25	0
5. <i>V. fasciculata</i> (V263)	x <i>V. membranacea</i> (V5)	23	4	1
6. <i>V. fasciculata</i> (V294)	x <i>V. sicula</i> (V365,V379)	33	5	0
7. <i>V. membranacea</i> (V5,V65)	x <i>V. fasciculata</i> (V294)	125	27	2

All hybrids counted proved to be triploid.

TABLE 6 Hybridisation results for *Vulpia-Festuca* intergeneric crosses.

Diploid-hexaploid combinations		No. of florets pollinated	No. of caryopses formed	Total Sells
Female	Male			
1. <i>F. rubra</i> (F11, F43, F70, F78, F89, F90)	x <i>V. sicula</i> (V365)	354	33	2
2. <i>V. alopecuroides</i> (V33)	x <i>F. rubra</i> (F6, F43, F51)	104	0	
3. <i>V. bromoides</i> (V110, V303)	x <i>F. rubra</i> (F31, F35, F37, F43, F45, F51)	91	7	7
4. <i>V. fontquerana</i> (V404)	x <i>F. rubra</i> (F44)	4	0	
5. <i>V. geniculata</i> (V356)	x <i>F. rubra</i> (F29)	75	0	
g. <i>V. ligustica</i> (V255, V257)	x <i>F. rubra</i> (F7, F43)	74	11	0
7. <i>V. membranacea</i> (V5, V423)	x <i>F. rubra</i> (F7, F35, F37, F43, F44, F45)	224	8	0
8. <i>V. sicula</i> (V365)	x <i>F. rubra</i> (F56, F71)	201	12	0
All hybrids counted proved to be tetraploid except the two hybrids F43 x V365, which were both heptaploid (2n = 49)				
Diploid-octoploid combinations				
1. <i>V. alopecuroides</i> (V33)	x <i>F. juncifolia</i> (F21B)	51	0	
2. <i>V. sicula</i> (V365)	x <i>F. juncifolia</i> (F21B, F41)	100	30	0
3. <i>V. sicula</i> (V365)	x <i>F. rubra</i> (F39)	404	64	0
All hybrids counted proved to be pentaploid.				
Tetraploid-hexaploid combination				
1. <i>V. fasciculata</i> (V294)	x <i>F. rubra</i> (F6, F31, F35, F44, F45)	90	27	0
All hybrids counted proved to be pentaploid				
Hexaploid-hexaploid combination				
1. <i>V. myuros</i> (V198)	x <i>F. rubra</i> (F35, F37, F43)	67	9	2
All hybrids counted proved to be hexaploid				

TABLE 7 Hybridisation results for interspecific *Festuca* crosses.

Hexaploid-octoploid combinations		No. of florets pollinated	No. of caryopses formed	
Female	Male		Total	Selfs
1. <i>F. juncifolia</i> (F3,F21B)	<i>F. rubra</i> (F11)	154	36	0
2. <i>F. rubra</i> subsp. arenaria (F21A)	<i>F. rubra</i> subsp. rubra (F11)	30	6	0

All hybrids counted were heptaploid.

reciprocals between the four species were attempted because the anthers were large and easy to emasculate and because they produced plenty of pollen for cross-pollination. Within section Monachne, V. membranacea x V. fasciculata and the reciprocal were attempted, but comparatively few crosses were made because the anthers were smaller and produced much less pollen for cross-pollination. V. fontquerana was more difficult than the other two species to emasculate because the lemma was more delicate and therefore easily damaged, allowing the whole floret to become desiccated. Within section Vulpia very few crosses were attempted as pollen production was very low (about 60 grains were counted in one anther) and it was very difficult to remove a ripe anther without bursting it. Emasculation within this section is also difficult as the anther and the filament are very small and in V. myuros and V. ciliata the inflorescence is completely enclosed within the sheath during self-pollination. Species in sections Monachne and Vulpia were mostly used as female parents and pollinated with species of section Loretia.

A total of 7,748 florets were cross-pollinated in 47 species combinations (including reciprocals). Figs. 22 and 23 show the species combinations and reciprocals attempted in the hybridisation experiments. 884 (11.4%) caryopses were produced. Many of the Vulpia interspecific hybrid caryopses were smaller than normal Vulpia caryopses and wrinkled. The interspecific fescue hybrid caryopses were hard, but smaller than normal fescue caryopses. The caryopses of the intergeneric hybrids varied in their degree of fullness.

ii. Embryo culture

The hybridisation experiments resulted in caryopses being produced from 27 different crosses (including reciprocals). All of these

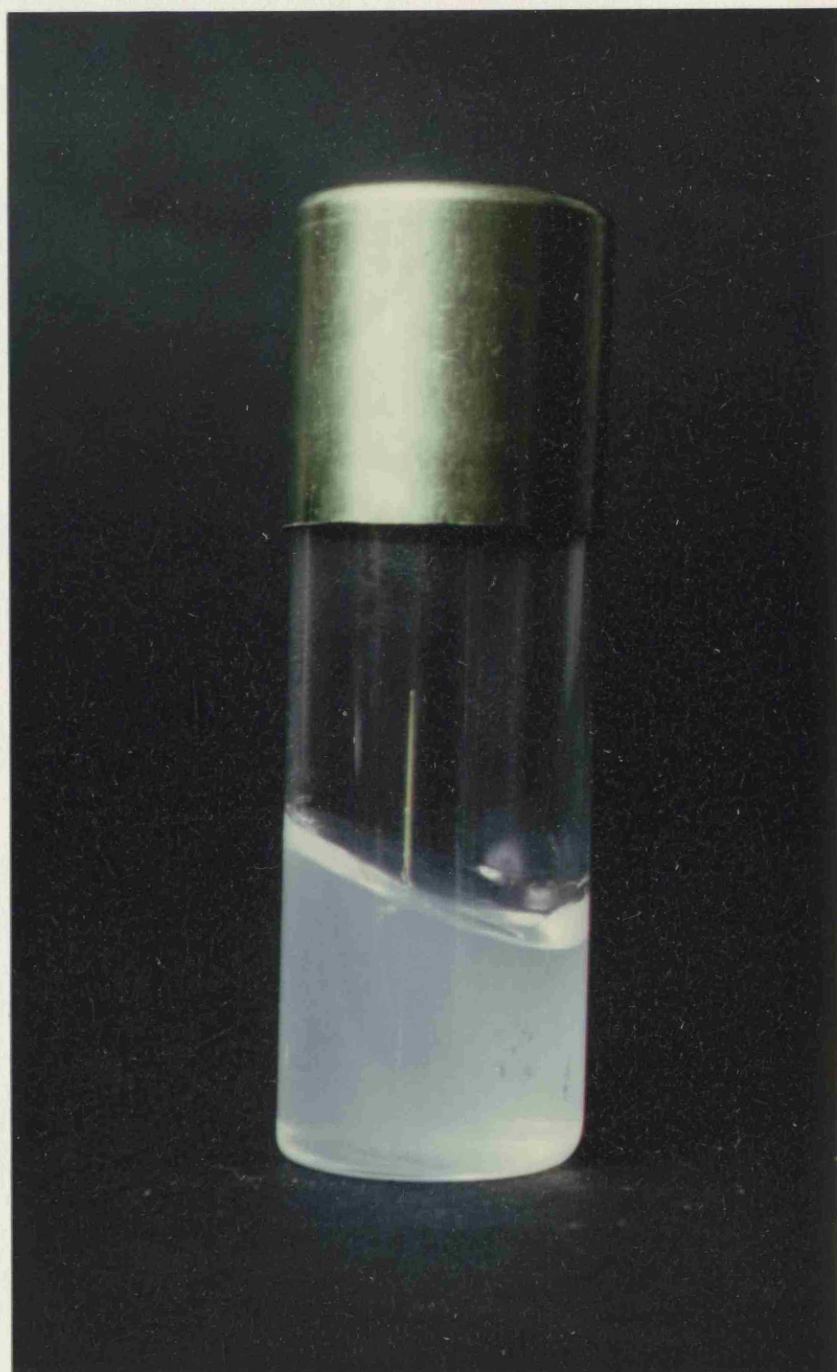
TABLE 8 Embryo culture results

Crosses	No. of embryos in embryo culture (nos. of caryopses with no embryos)	No. of seedlings	No. of mature plants	Chromosome number	% success using embryo culture	Size of embryo	Type of endosperm
<u>Interspecific Vulpia crosses</u>							
<u>Diploid-diploid</u>							
V. alopecuroides x V. membranacea	3	1	0		0%		Watery
V. fontquerana x V. alopecuroides	4 (8)	0			0%		Watery
V. fontquerana x V. ligustica	2 (2)	2	2	14	50%	Medium	Hard
V. geniculata x V. sicula	1 (1)	0			0%	Small	Watery
V. ligustica x V. geniculata	84 (57)	22	17	14	10%	Small	Soft or watery
V. ligustica x V. sicula	1	0			0%	Small	Watery
V. membranacea x V. alopecuroides	29 (14)	0			0%	Small	Watery
V. membranacea x V. geniculata	24 (10)	9	9	14	41%	Large	Hard or soft
V. membranacea x V. ligustica	43	31	10	14	32%	Large	Mixed
V. membranacea x V. sicula	12	8	5	14	41%	Large	Hard
V. sicula x V. geniculata	60 (80)	5	1	14	0.7%	Small	Watery
<u>Diploid-tetraploid</u>							
V. fasciculata x V. alopecuroides	1	1	1	21	100%	Large	Hard
V. fasciculata x V. geniculata	13 (2)	4	4	21	27%		Hard
V. fasciculata x V. ligustica	17 (8)	10	10	21	40%		Hard
V. fasciculata x V. membranacea	2 (1)	1	1	21	33%		Hard
V. fasciculata x V. sicula	4 (1)	3	3	21	60%		Hard
V. membranacea x V. fasciculata	16 (10)	7	7	21	27%		Hard
<u>Intergeneric Vulpia-Festuca crosses</u>							
<u>Diploid-hexaploid</u>							
F. rubra x V. sicula	25 (6)	20	20	*	65%	Medium-large	Hard
V. ligustica x F. rubra	2 (9)	0			0%		
V. membranacea x F. rubra	2 (6)	0			0%		
V. sicula x F. rubra	9 (3)	1	1	28	8%	Small	Soft
<u>Diploid-octoploid</u>							
V. sicula x F. juncifolia	30	3	1	35	3%		
V. sicula x F. rubra	64	2	2	35	3%	Medium	Soft
<u>Tetraploid-hexaploid</u>							
V. fasciculata x F. rubra	25 (2)	23	23	35	85%		Hard
<u>Hexaploid-hexaploid</u>							
V. myuros x F. rubra	7	2	2	42	29%		
<u>Interspecific Festuca crosses</u>							
<u>Hexaploid-octoploid</u>							
F. juncifolia x F. rubra	36 (3)	23	16	49	41%	Large	Hard
F. rubra subsp. arenaria x F. rubra subsp. rubra	6	6	3	49	50%	Large	Hard

* Two plants had $2n = 49$, the rest counted had $2n = 28$.

Plate 2 Seedling germinated using the embryo culture technique.

Plate 2



caryopses were used for embryo culture and hybrid plants were obtained for 20 different crosses (Table 8).

Germination of the embryos on the agar took from 5 to 21 days, and most of the embryos germinated within the first ten days. A few embryos developed into a light-brown coloured callus. Some other embryos germinated but lacked chlorophyll and only grew about 2 cm. Several seedlings had vigorous leaf growth but insufficient root growth and when they were transferred to soil they died. A few other seedlings died when transplanted to soil; this may have been due to excess moisture. About 20 vials of agar had had fungal infection through faulty technique, but in some cases it was possible to save the seedling.

The results of the embryo culture experiments are presented in Table 8. Of the 884 caryopses used in embryo culture, 223 caryopses when dissected had no obvious embryo (due to the embryo not developing or its disintegration from lack of endosperm); this number is given in brackets for individual crosses in Table 8. Embryos could be dissected from 661 caryopses and from these 138 mature hybrid plants were obtained. The size of the embryo and the condition of the endosperm is also noted in Table 8. Plate 2 shows a seedling germinated from embryo culture and growing in agar. Figs. 22 and 23 show those crosses in which caryopses were formed but did not germinate, and those in which the caryopses germinated and grew to mature hybrid plants.

iii. Hybrid plants

The hybrid nature of the plants resulting from crosses between parental species having the same chromosome number were checked by morphological observations. The total number of selfs from all the hybridisation experiments was 20.

All the hybrids flowered in the following year after they

had been germinated by embryo culture, except plants from four different crosses. The V. sicula x F. rubra and F. rubra x F. juncifolia hybrids made in 1978 failed to flower in 1979. Of the F. rubra x V. sicula hybrids also made in 1978, all failed to flower in 1979 except two plants, of which one bore two inflorescence and the other a single inflorescence. The 23 V. fasciculata x F. rubra hybrids made in 1977 failed to flower in the summer of 1978 and were given a cold treatment in the autumn of that year (5°C and short days for three months in a growth cabinet). After the cold treatment about half of the hybrids flowered and the next summer all of them did so.

The time of day was found to be very important when picking inflorescences for meiosis. Pollen mother cell meiosis in the parents and the hybrids was found to occur about noon in midsummer and as late as 3.30 p.m. in mid-winter.

The start of meiosis was determined by the length of emergence of the inflorescence from the sheath. This critical degree of emergence varied from section to section: in section Loretia the inflorescence was $\frac{3}{4}$ emerged from the sheath; in section Monachne just the awns of the topmost spikelets protruded from the sheath; in V. myuros the inflorescence was completely enclosed within the sheath; and in F. rubra the inflorescence was $\frac{1}{2}$ - $\frac{3}{4}$ emerged from the sheath. Meiosis in the hybrids occurred when inflorescence emergence was roughly intermediate between that of the parental species.

For each cross, pressed herbarium specimens were taken for most hybrid plants and a few plants were left to see whether any F_2 caryopses would develop. For each pressed specimen of a hybrid plant ten samples of each inflorescence were measured (except for callus length where smaller samples were measured) and the mean was calculated for each plant. In the tables of hybrid plant measurements the ranges of means are given. Exceptions to this are where there is only one hybrid plant

from a cross and then the mean and the range of measurements from the single plant are given. The ranges of pollen grain size are means of 50 samples from several hybrid plants. The measurements given for parental species are taken from two or three different seed accessions for each species used in crossing, (except for V. fontquerana and V. sicula where V404 and V365 respectively were used) and these plants were grown under the same conditions as the hybrids and at the same time of year. For three crosses, i.e. V. ligustica x V. geniculata, V. membranacea x V. geniculata and V. membranacea x V. ligustica, a sufficient number of hybrid plants ~~was~~ available for a statistical analysis of variance to be undertaken, to compare the inflorescence characters of the hybrids with those of the mean of the parental species.

The table of performance of F_1 artificial hybrids is given in Table 30. The case histories of the hybridisation attempts for each of the 46 species combinations are given below.

iv. Case histories of hybridisation attempts for individual cross combinations

a. Interspecific Vulpia crosses

Diploid-diploid combinations

1. V. alopecuroides x V. geniculata

435 florets were pollinated but no caryopses were produced. See No. 10 for reciprocal.

2. V. alopecuroides x V. ligustica

465 florets were pollinated but no caryopses were produced. See No. 14 for reciprocal.

3. V. alopecuroides x V. membranacea

127 florets were pollinated and three caryopses were formed. One of these germinated in embryo culture, but died as a young seedling.

See No. 18 for reciprocal.

4. V. alopecuroides x V. sicula

266 florets were pollinated but no caryopses were produced. See No. 22 for reciprocal.

5. V. bromoides x V. sicula

Twelve florets were pollinated but no caryopses were produced.

6. V. fontquerana x V. alopecuroides

95 florets were pollinated and 13 caryopses were formed. One caryopsis germinated but was a self; the rest did not germinate in embryo culture.

7. V. fontquerana x V. geniculata

16 florets were pollinated but no caryopses were produced.

8. V. fontquerana x V. ligustica

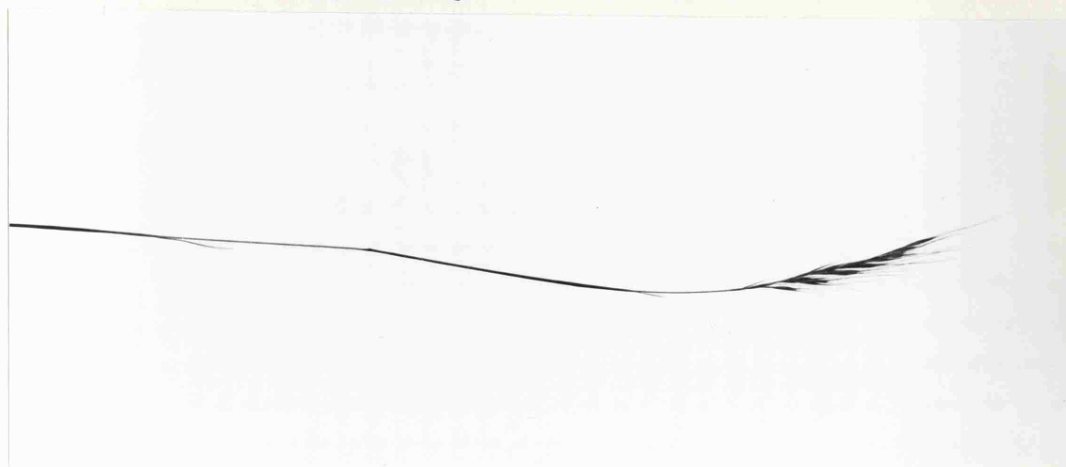
106 florets were pollinated and four caryopses were produced. Embryos were only found in two caryopses. These germinated in embryo culture and grew to mature plants. The hybrids took six months to flower.

The plants were small and not very vigorous, and not many inflorescences were produced.

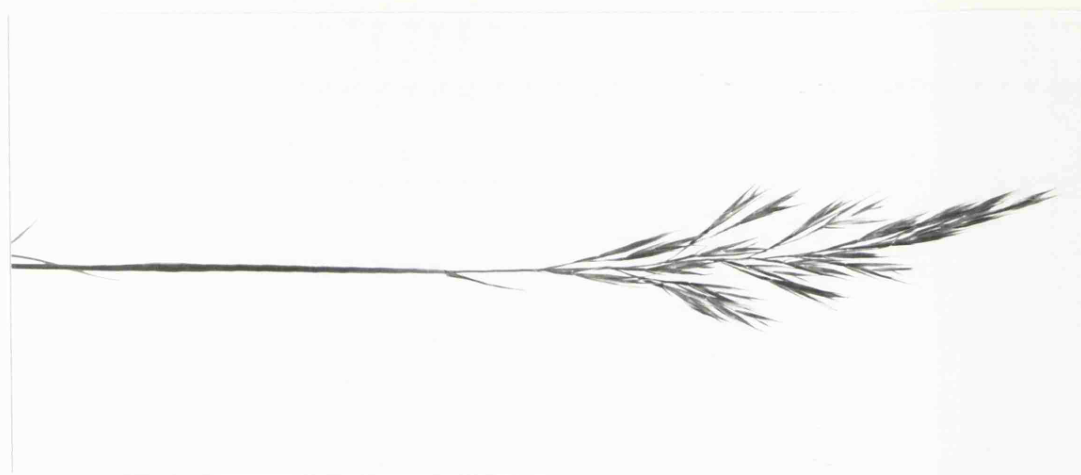
The hybrids were intermediate between the parental species in general appearance. The inflorescence was branched with several spikelets growing from some nodes, but not so branched as in V. ligustica

- Plate 3
- a) Inflorescence of V. fontquerana (x 0.6)
 - b) Inflorescence of V. fontquerana x V. ligustica artificial hybrid (x 0.7)
 - c) Inflorescence of V. ligustica (x 0.8)
 - d) P.M.C. of V. fontquerana x V. ligustica at anaphase I (x 1,800)
 - e) Micronuclei in young pollen grains of V. fontquerana x V. ligustica (x 1,800)

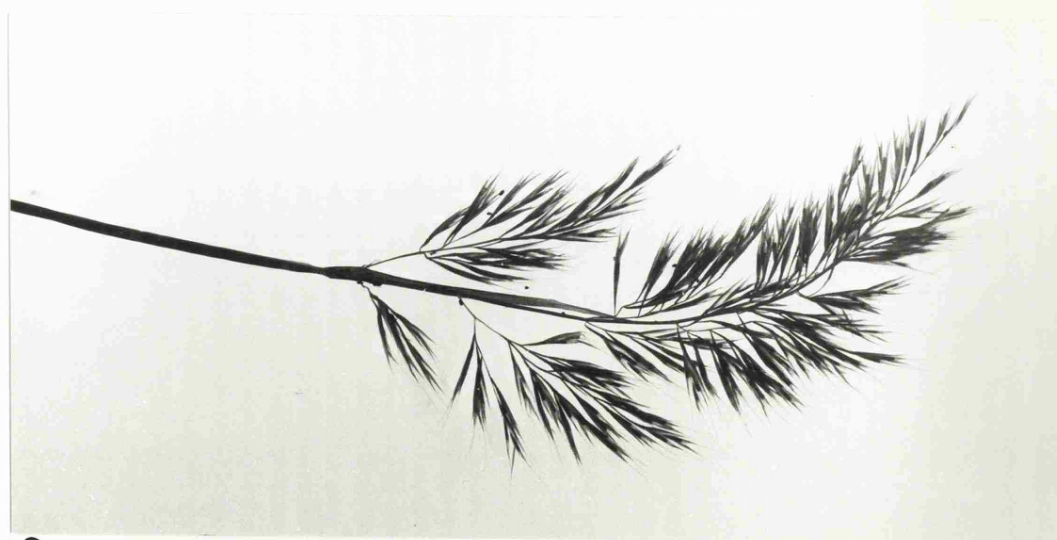
Plate 3



a



b



c

Plate 3 (cont.)



d



e

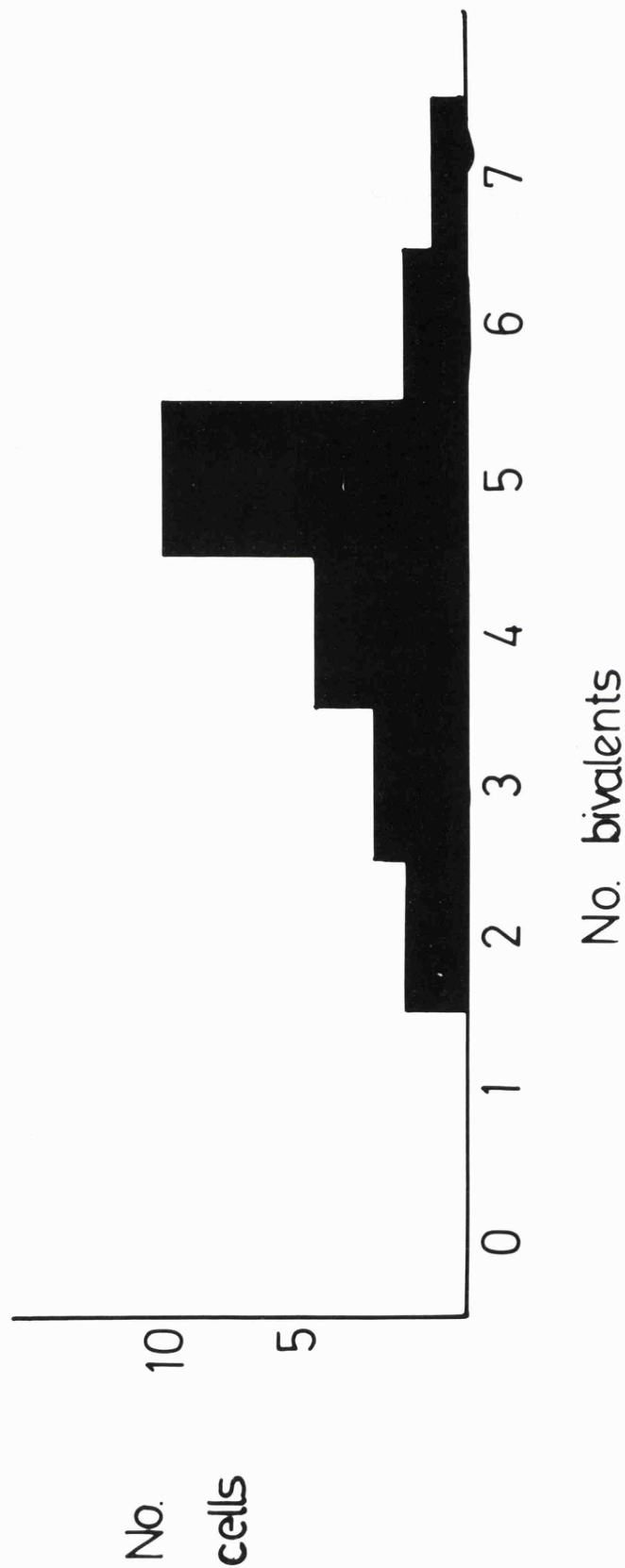
TABLE 9 Measurements of inflorescence characters for V. fontquerana, V. ligustica and
V. fontquerana x V. ligustica hybrids.

Inflorescence characters	<u>V. fontquerana</u>	Hybrid	<u>V. ligustica</u>
Lower glume length mm	0.6 - 4.0	1.2 - 2.7	0.2 - 0.7
Upper glume length (-awn) mm	4.0 - 9.0	4.4 - 6.8	6.6 - 10.8
Glume awn length mm	1.0 - 6.0	2.0	0 - 2.7
Lemma length (-awn) mm	4.0 - 8.0	4.5 - 5.7	5.4 - 7.8
Lemma awn length mm	11.0 - 18.0	5.0 - 7.2	2.7 - 6.6
Glume ratio	$\frac{1}{4} - \frac{1}{2}$	$\frac{1}{10} - \frac{2}{3}$	$< \frac{1}{10}$
Anther length mm	0.5 - 0.8	0.5 - 1.2	1.8 - 2.6
Pedicel length mm	2.0 - 5.8	3.4	3.1 - 5.1
Callus length mm	1.0 - 1.5	0.4	0.2
Ovary, glabrous or pubescent	G	P	P
Chromosome number	14	14	14

KEY

G glabrous
P pubescent

FIGURE 1. HISTOGRAM OF NUMBER OF BIVALENTS
OBSERVED AT MEIOSIS IN V. FONTQUERANA X
V. LIGUSTICA HYBRIDS.



(Plate 3). The hybrids had purple awns like those of V. fontquerana and the whole inflorescence turned purple when mature, unlike either parental species.

The hybrid was intermediate for all characters measured, except callus length and lemma awn length, both of which were closer to the measurement for V. ligustica (Table 9). The ovary tip was pubescent as in V. ligustica.

23 cells were observed at pollen mother cell (PMC) meiosis (Plate 3). The number of bivalents ranged from two to seven, the mode was five bivalents and the mean was 4.4 bivalents (Fig. 1). No multivalents were observed and the mean number of chiasmata per cell was 6.09. The pollen was 100% sterile and the anthers were exerted during flowering but were indehiscent. The hybrids were seed sterile.

9. V. fontquerana x V. sicula

37 florets were pollinated but no caryopses were produced.

10. V. geniculata x V. alopecuroides

111 florets were pollinated but no caryopses were produced. See No. 1 for reciprocal.

11. V. geniculata x V. ligustica

200 florets were pollinated but no caryopses were produced. See No. 15 for reciprocal.

12. V. geniculata x V. membranacea

72 florets were pollinated but no caryopses were produced. See No. 19 for reciprocal.

13. V. geniculata x V. sicula

295 florets were pollinated and two caryopses were produced. Neither of these germinated in embryo culture. See No. 23 for reciprocal.

14. V. ligustica x V. alopecuroides

78 florets were pollinated but no caryopses were produced. See No. 2 for reciprocal.

15. V. ligustica x V. geniculata

212 florets were pollinated and 141 caryopses were produced. Embryos were not found in 57 of the caryopses. Of the 84 embryos cultured, 22 germinated and 17 of these grew to mature plants.

The hybrids flowered about two months after germination. All the plants were vigorous and produced many inflorescences. The hybrids were intermediate in general appearance between the parental species (Plate 4).

Pressed specimens were made of nine hybrid plants. A statistical analysis of variance was carried out to compare the hybrids with the parents for the following five characters.

Lower glume length - the hybrids were not significantly different from the mean of the parental species and were therefore intermediate for this character.

Upper glume length (-awn) - the hybrids were not significantly different from the mean of the parental species and was therefore intermediate for this character.

Lemma length (-awn) - the parental species were not significantly different for this character.

Plate 4 a) Inflorescence of V. geniculata (x 0.6)

b) Inflorescence of V. ligustica x V. geniculata artificial hybrid (x 0.5).

See Plate 3c for inflorescence of V. ligustica.

c) Floret of V. ligustica x V. geniculata hybrid at anthesis with anthers and stigmas exerted (x 5)

d) P.M.C. of V. ligustica with seven bivalents at metaphase I (x 2,500)

e) P.M.C. of V. ligustica x V. geniculata with seven bivalents at metaphase I (x 2,500)

f) P.M.C. of V. ligustica x V. geniculata at anaphase (x 2,000)

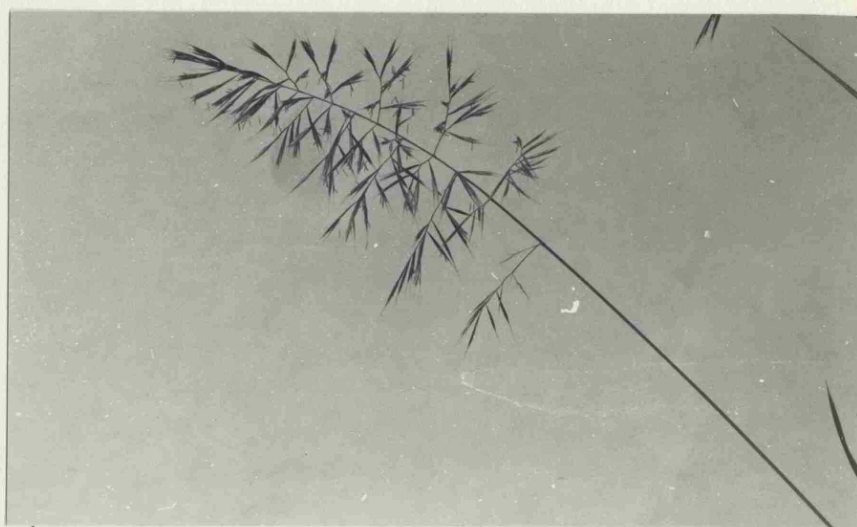
g) P.M.C. of V. ligustica x V. geniculata at telophase (x 2,000)

h) Pollen grains of V. ligustica x V. geniculata (x 400)

Plate 4



a

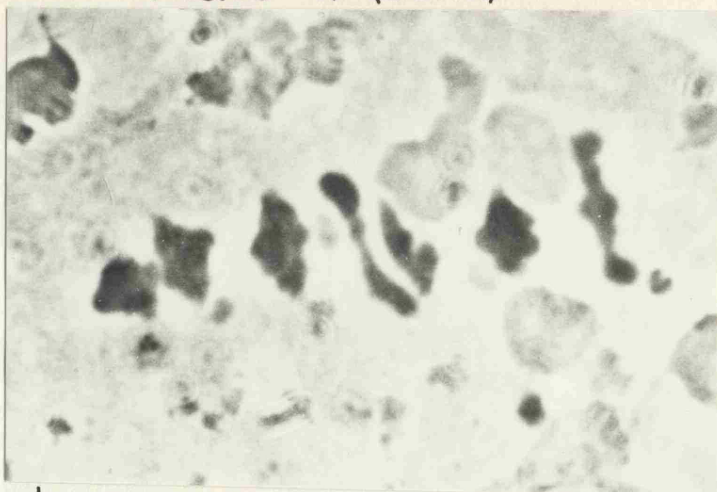


b



c

Plate 4 (cont.)



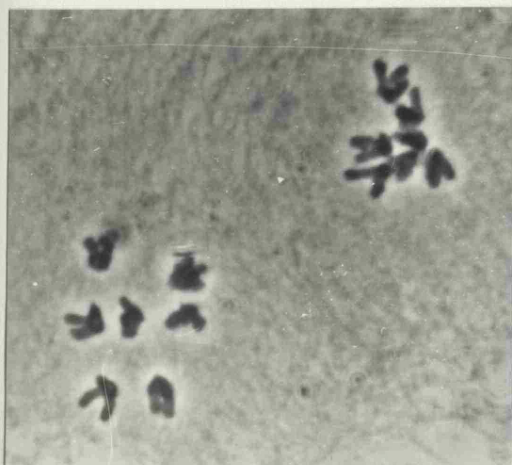
d



e



f



g



h

TABLE 10 Measurements of inflorescence characters for V. ligustica, V. geniculata and
V. ligustica x V. geniculata hybrids

Inflorescence characters	<u>V. ligustica</u>	Hybrid	<u>V. geniculata</u>
Lower glume length mm	0.2 - 0.7	1.3 - 3.3	2.3 - 4.5
Upper glume length (-awn) mm	6.6 - 10.8	6.8 - 9.9	5.4 - 8.1
Upper glume awn length mm	0 - 2.7	0 - 1.8	0 - 0.4
Lemma length (-awn) mm	5.4 - 7.8	5.6 - 7.3	4.3 - 7.4
Lemma awn length mm	2.7 - 6.6	4.0 - 7.1	2.6 - 4.5
Glume ratio	$< \frac{1}{10}$	$\frac{1}{6} - \frac{1}{2}$	$\frac{2}{5} - \frac{3}{5}$
Anther length mm	1.8 - 2.6	1.6 - 2.4	1.9 - 2.7
Pedicel length mm	3.1 - 5.1	3.5 - 5.6	4.1 - 7.6
Callus length mm	0.2	0.2 - 0.3	0.2
Ovary glabrous or pubescent	P	P	P
Pollen grain size μ m	27 - 29	20 - 25	26 - 29
Chromosome number	14	14	14

FIGURE 2. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V. LIGUSTICA, V. GENICULATA AND HYBRIDS.

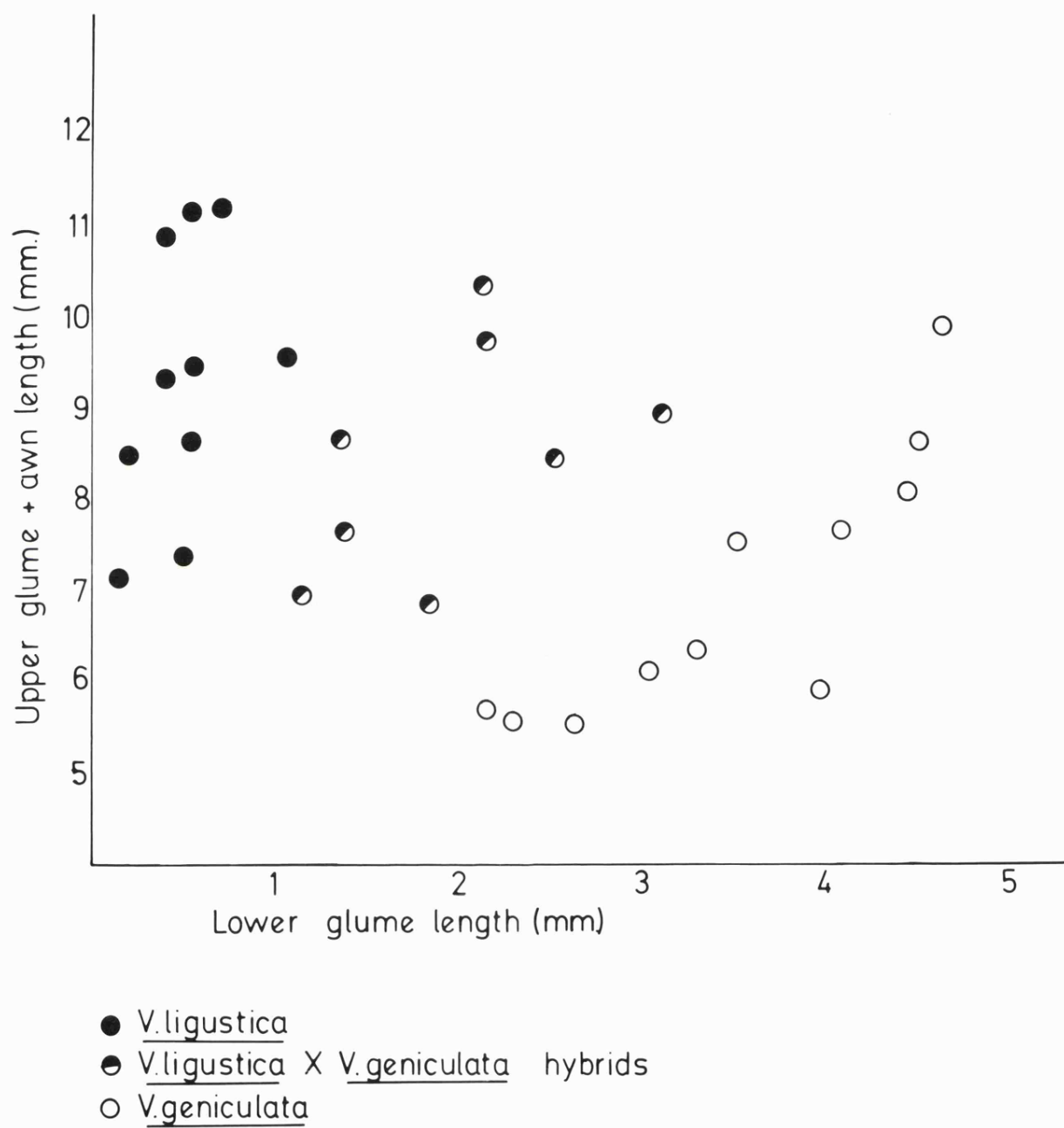
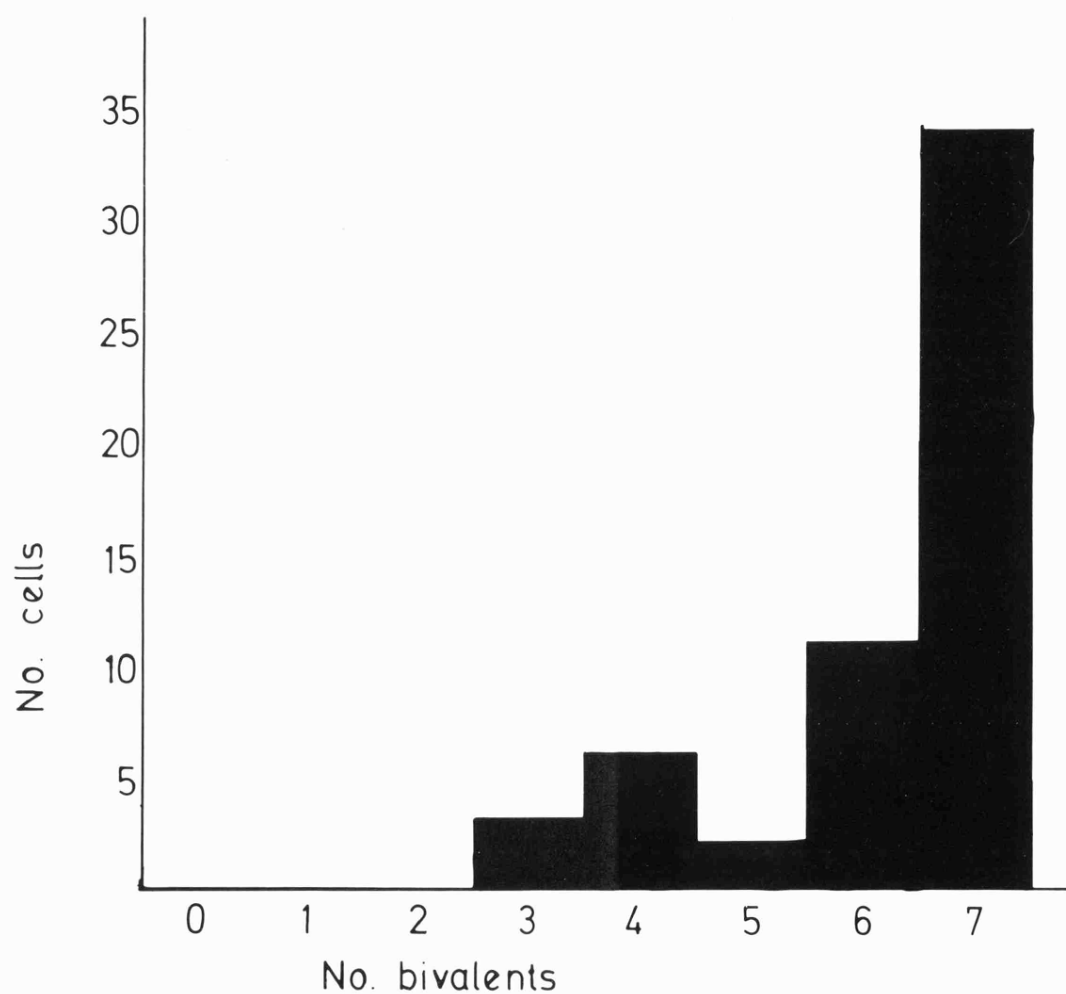


FIGURE 3. HISTOGRAM OF NUMBER OF BIVALENTS OBSERVED AT MEIOSIS IN V. LIGUSTICA X V. GENICULATA HYBRIDS.



Lemma awn length - the hybrids were significantly different ($P = 0.01$) from the mean of the parental species and were closer to V. ligustica.

Anther length - no significant difference was found between the parental species or between hybrids and parents for this character.

The hybrids had smaller pollen grains than both parents (Table 10). Fig. 2 is a scatter diagram of inflorescence characters of the parents and hybrids showing the intermediacy of the hybrids.

56 cells were observed at meiosis. The number of bivalents ranged from two to seven, the mode (61% of the cells) was seven bivalents (Fig. 3) and the mean was 6.2 bivalents. No multivalents were observed and four to six ring bivalents were formed (Plate 4). A few cells were observed at diakinesis with chromosomes lying together in pairs but with no visible chiasmata, though in most cells seven bivalents were observed and at anaphase the chromosomes separated normally (Plate 4). In one particular hybrid the later stages of meiosis were studied. At first division telophase half the cells had laggards and at the tetrad stage 53% of the tetrads had micronuclei. The pollen sterility in this particular hybrid was 98%. Pollen sterility in other hybrids varied from 100% (and in some cases no pollen was present at all) to 99% sterility. The florets opened and the anthers were well exerted during flowering but the anthers were indehiscent and all the hybrids were seed sterile. See No. 11 for reciprocal.

16. V. ligustica x V. membranacea

56 florets were pollinated but no caryopses were produced. See No. 20 for reciprocal.

17. V. ligustica x V. sicula

216 florets were pollinated and one caryopsis was produced. The

embryo did not germinate in embryo culture. See No. 24 for reciprocal.

18. V. membranacea x V. alopecuroides

197 florets were pollinated and 43 caryopses were produced. In 14 caryopses the embryo could not be found after dissection and none of the 29 cultured embryos germinated. See No. 3 for reciprocal.

19. V. membranacea x V. geniculata

208 florets were pollinated and 34 caryopses were produced. Of these ten embryos could not be found after dissection. Nine of the 24 embryos cultured germinated and all of these grew into mature plants.

The V. membranacea x V. geniculata hybrids took only two months to flower from germination. The hybrids were vigorous and produced many inflorescences. The hybrid plants were intermediate in general appearance, having a branched inflorescence but this being not as spreading as in V. geniculata (Plate 5).

Eight plants were pressed and a statistical analysis of variance was carried out for a comparison of parents and hybrids for the five following characters.

Lower glume length - the hybrids were significantly different ($P = 0.01$) from the mean of the parental species and were closer to V. geniculata.

Upper glume length (-awn) - the hybrids were significantly different ($P = 0.01$) from the mean of the parental species and were closer to V. membranacea.

Lemma length (-awn) - the hybrids were not significantly different from the mean of the parental species and were therefore intermediate for this character.

Plate 5 a) Inflorescence of V. membranacea (x 1.0)

b) Inflorescence of V. membranacea x V. geniculata artificial
hybrid (x 0.8)

See Plate 4a for inflorescence of V. geniculata.

Plate 5



a



b

TABLE 11 Measurements of inflorescence characters for V. membranacea, V. geniculata and
V. membranacea x V. geniculata hybrids

Inflorescence characters	V. membranacea	Hybrid	V. geniculata
Lower glume length mm	0.5 - 1.2	3.1 - 5.5	2.3 - 4.5
Upper glume length (-awn) mm	6.7 - 10.6	7.2 - 10.0	5.4 - 8.2
Upper glume awn length mm	1.1 - 3.6	0 - 1.1	0 - 0.4
Lemma length (-awn) mm	6.7 - 10.6	6.5 - 8.1	4.3 - 7.4
Lemma awn length mm	6.2 - 13.5	6.3 - 9.5	2.6 - 4.5
Glume ratio	$< \frac{1}{10}$	$2 - \frac{1}{5}$	$2 - \frac{3}{5}$
Anther length mm	0.4 - 0.7	0.9 - 1.4	1.9 - 2.7
Pedicel length mm	2.3 - 5.4	4.1 - 7.0	4.1 - 7.6
Callus length mm	0.5 - 0.8	0.2 - 0.4	0.2
Ovary glabrous or pubescent	G	P	P
Pollen grain size μ m	27 - 31	17 - 27	26 - 29
Chromosome number	14	14	14

FIGURE 4. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V. MEMBRANACEA, V. GENICULATA AND HYBRIDS.

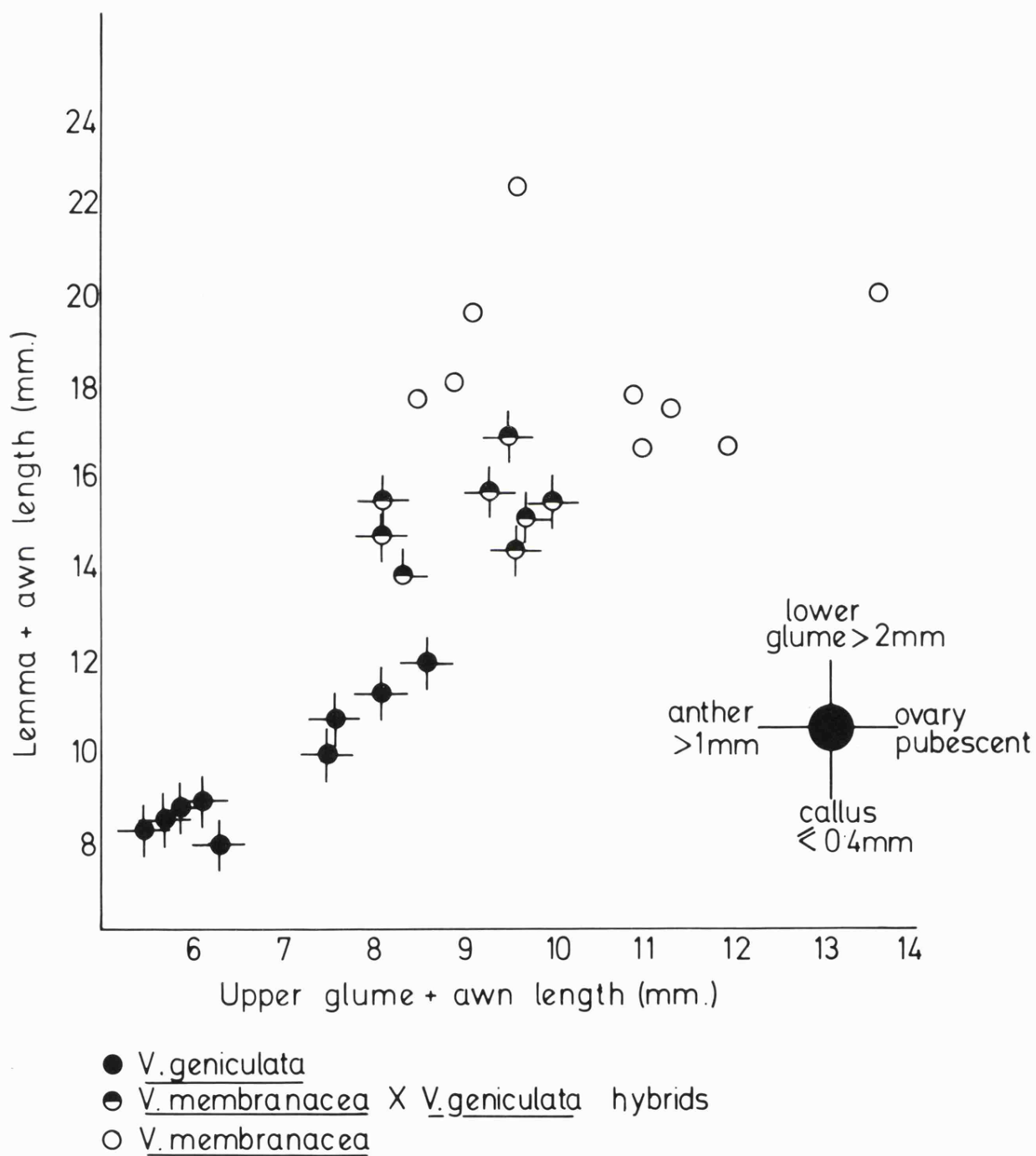
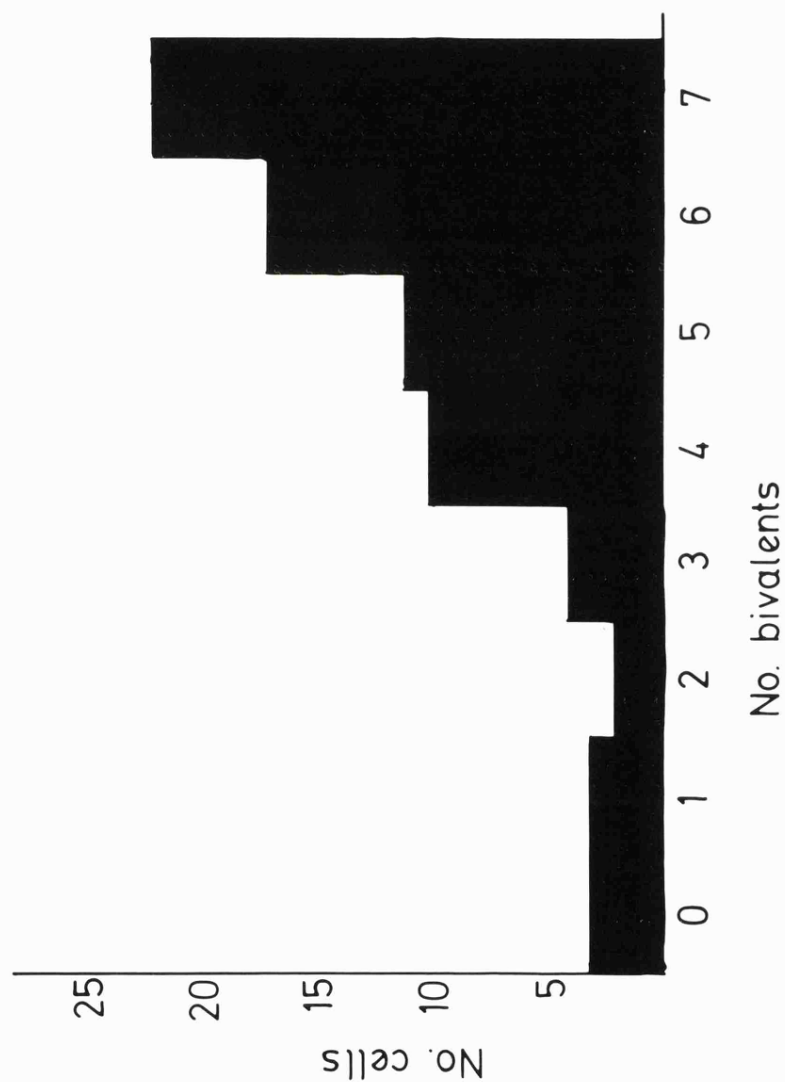


FIGURE 5. HISTOGRAM OF NUMBER OF BIVALENTS
OBSERVED AT MEIOSIS IN V. MEMBRANACEA X
V. GENICULATA HYBRIDS.



Anther length - the hybrids were significantly different ($P = 0.05$) from the mean of the parental species and were slightly closer to V. membranacea.

The hybrids had pubescent ovary tips like V. geniculata and had callus length intermediate between the two parental species (Table 11). Fig. 4 is a scatter diagram of inflorescence characters of the parents and hybrids showing the intermediacy of the hybrids.

72 cells were observed at meiosis. The number of bivalents ranged from none to seven, the mode was seven and the mean was 5.1 bivalents (Fig. 5). Mostly rod bivalents were observed and there were no multivalents. In 15% of the cells observed at diakinesis, some of the chromosomes were lying together in pairs but there were no visible chiasmata. The pollen in all the hybrids was 100% sterile and was smaller than in either parent. The florets opened during flowering and the anthers were exerted but the anthers were indehiscent. All the hybrids were seed sterile. See No. 12 for reciprocal.

20. V. membranacea x V. ligustica

242 florets were pollinated and 46 caryopses were produced but three of these were found to be selfs. Of the 43 hybrid caryopses, 31 germinated. Ten plants died at the seedling stage and eleven established plants died before flowering. Ten plants remained and all of these flowered after six months. The plants made a lot of vegetative growth before flowering and once flowering commenced it continued for up to ten months, until the plant died.

The inflorescence was a raceme with occasionally more than one spikelet at the lower nodes, and thus less branched than either parental species (Plate 6). The culm always grew erect as in V. ligustica. The lemma awns on some hybrids were slightly purple.

Plate 6 a) Inflorescence of V. ligustica (x 0.8)

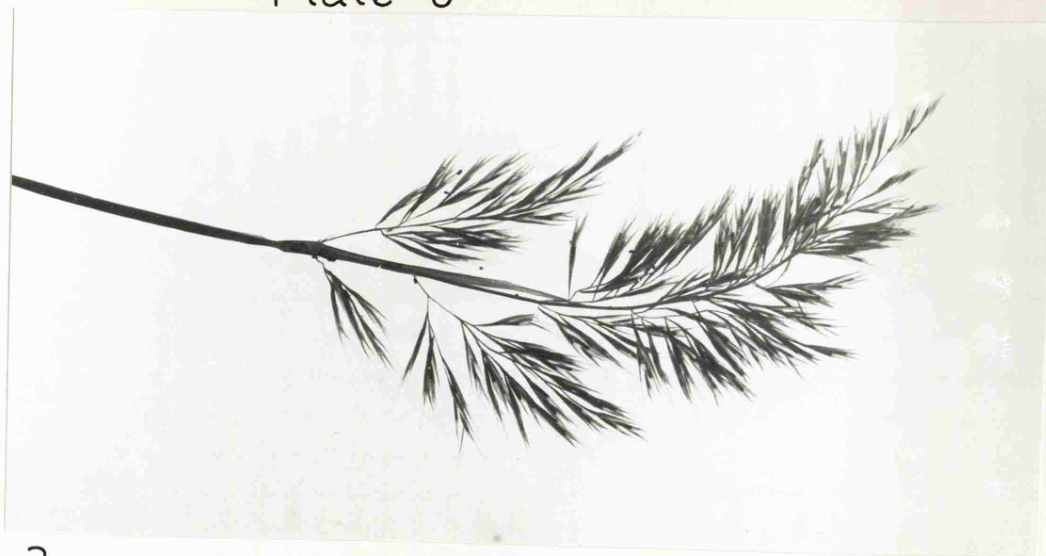
b) Inflorescence of V. membranacea x V. ligustica artificial
hybrid (x 1)

See Plate 5a for inflorescence of V. membranacea

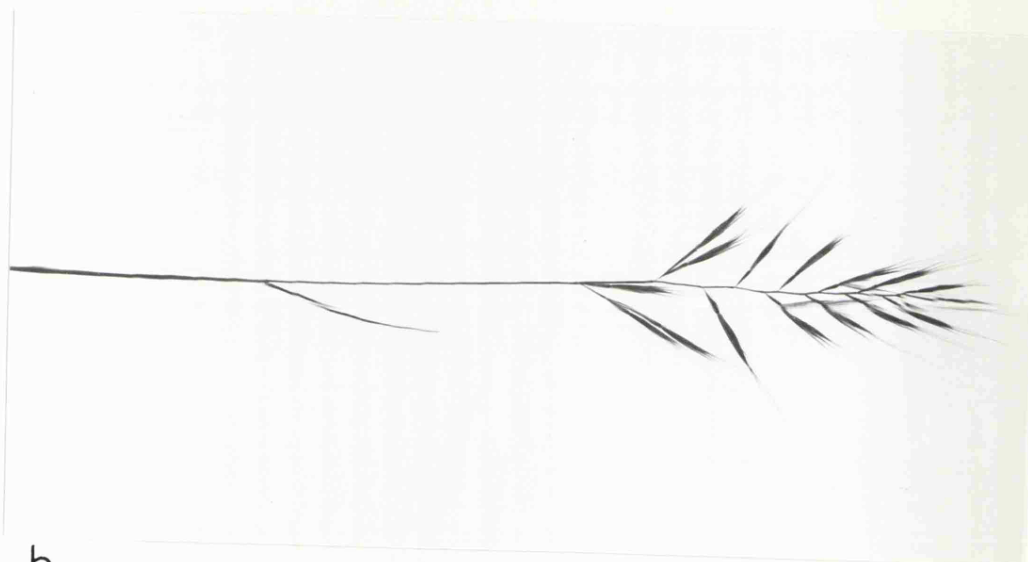
c) P.M.C. of V. membranacea x V. ligustica with seven bivalents
at metaphase I (x 2,500)

d) Abnormal division and micronuclei at the tetrad stage of
meiosis in V. membranacea x V. ligustica (x 400)

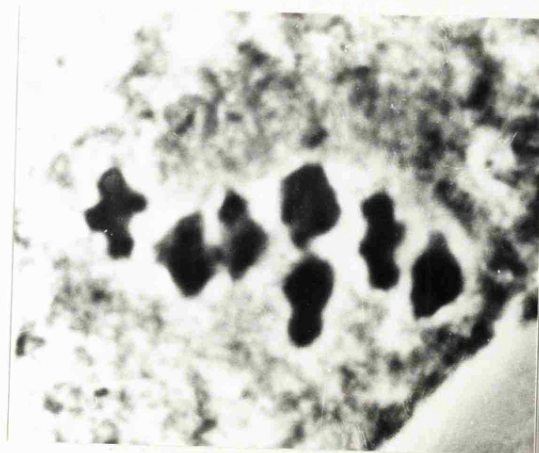
Plate 6



a



b



c



d

Eight plants were pressed and a statistical analysis of variance was carried out comparing the following five inflorescence characters of the parental species and the hybrids.

Lower glume length - the hybrids were not significantly different from the mean of the parental species and were therefore intermediate.

Upper glume length (-awn) - no significant difference was found between the parental species.

Lemma length (-awn) - the hybrids were not significantly different from the mean of the parental species and were therefore intermediate for this character.

Lemma awn length - the hybrids were significantly different ($P = 0.05$) from the mean of the parental species and were slightly closer to V. ligustica.

Anther length - the hybrids were significantly different ($P = 0.05$) from the mean of the parental species and were slightly closer to V. membranacea.

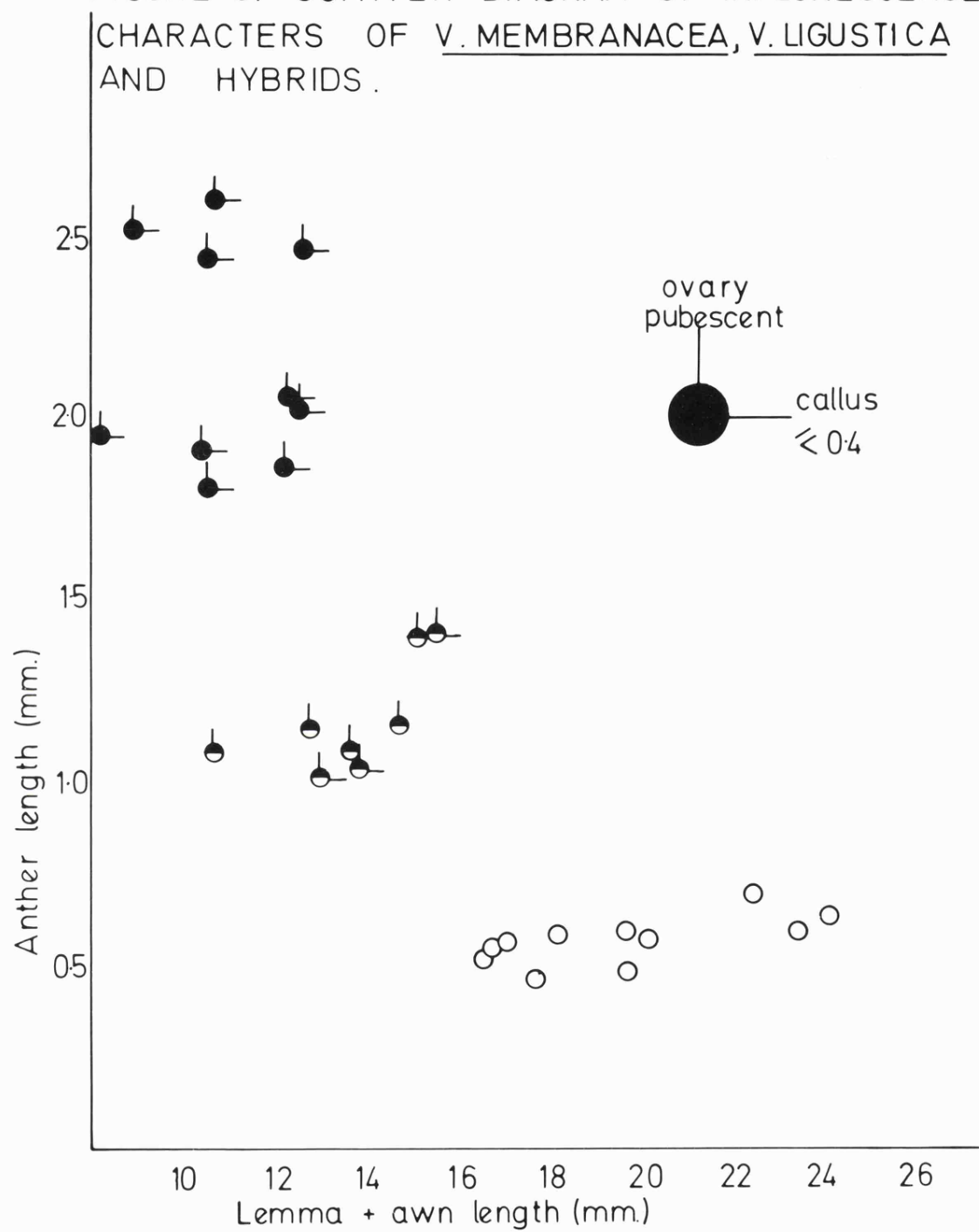
The hybrids were also intermediate for callus length and had pubescent ovary tips like V. ligustica (Table 12). Fig. 6 is a scatter diagram of inflorescence characters of parents and hybrids and shows the intermediacy of the hybrids.

97 cells were observed at meiosis and the number of bivalents ranged from one to seven and the mean number of bivalents was 4.9 (Table 13). The number of bivalents (including trivalents) had a mode of six bivalents (Fig. 7, Plate 6). Four cells had a trivalent and the mean number of chiasmata per cell was 7.63. The pollen in all the hybrids was 100% sterile and was empty and crumpled. The florets opened

TABLE 12 Measurements of inflorescence characters for V. membranacea, V. ligustica and
V. membranacea x V. ligustica hybrids

Inflorescence characters	<u>V. membranacea</u>	Hybrid	<u>V. ligustica</u>
Lower glume length mm	0.5 - 1.2	0.6 - 1.2	0.2 - 0.7
Upper glume length (-awn) mm	6.7 -10.6	6.9 -11.6	6.6 -10.8
Upper glume awn length mm	1.1 - 3.6	0 - 2.9	0 - 2.7
Lemna length (-awn) mm	6.7 -10.6	6.0 - 8.7	5.4 - 7.8
Lemna awn length mm	6.2 -13.5	4.2 - 8.2	2.7 - 6.6
Glume ratio	$< \frac{1}{10}$	$< \frac{1}{10}$	$< \frac{1}{10}$
Anther length mm	0.4 - 0.7	1.0 - 1.4	1.9 - 2.6
Pedicel length mm	2.3 - 5.4	2.8 - 5.3	3.1 - 5.1
Callus length mm	0.5 - 0.8	0.3 - 0.5	0.2
Ovary pubescent or glabrous	G	P	P
Chromosome number	14	14	14

FIGURE 6. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V. MEMBRANACEA, V. LIGUSTICA AND HYBRIDS.

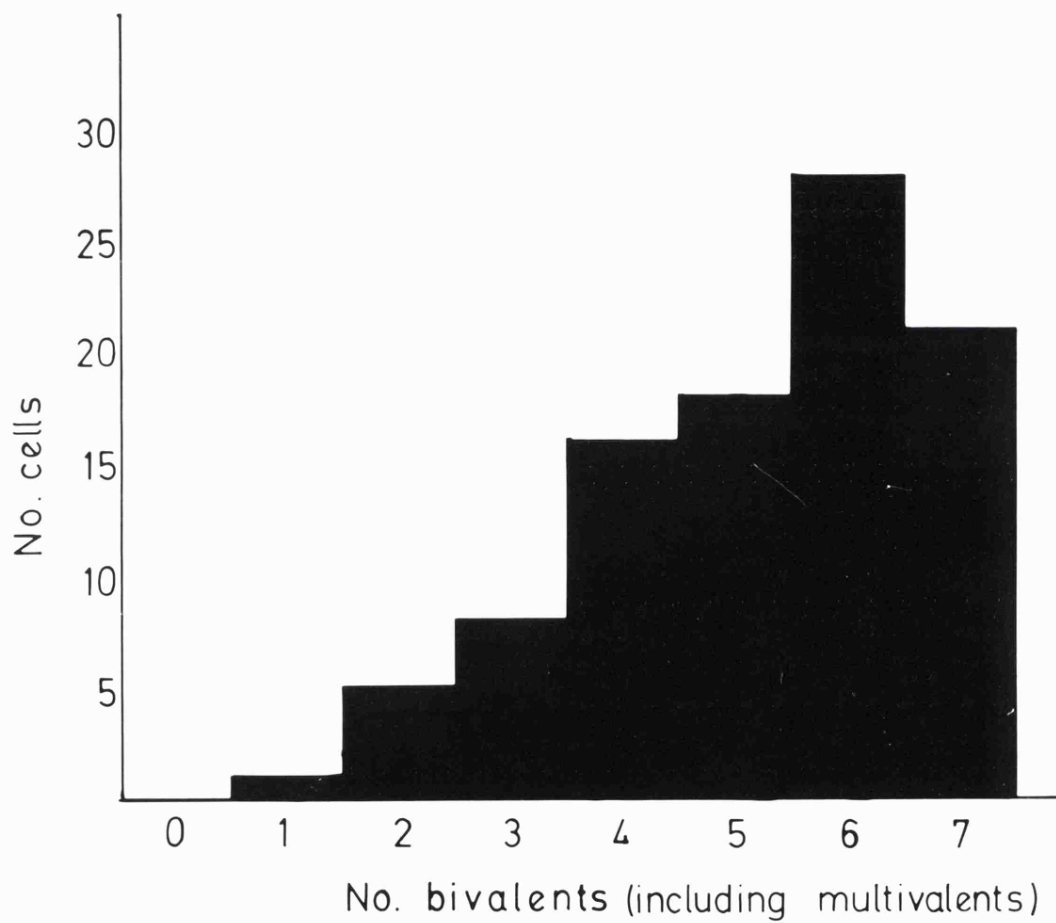


- V. membranacea
- ◐ V. membranacea X V. ligustica hybrids
- V. ligustica

TABLE 13 Chromosome associations at meiosis in V. membranacea x
V. ligustica hybrids

No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
	7	0	21
	6	2	27
1	5	1	1
	5	4	17
1	4	3	1
	4	6	15
1	3	5	1
	3	8	7
1	2	7	1
	2	10	5
	1	12	1

FIGURE 7. HISTOGRAM OF NUMBER OF BIVALENTS (INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN V. MEMBRANACEA X V. LIGUSTICA HYBRIDS.



during flowering and the anthers were exerted but they were indehiscent and the hybrids were seed sterile. See No. 16 for reciprocal.

21. V. membranacea x V. sicula

297 florets were pollinated and 13 caryopses grew. One caryopsis was later found to be a self. Of the twelve hybrid caryopses, eight embryos germinated in embryo culture and five of the seedlings grew to mature plants.

These hybrids took a long time to flower; the earliest flowered after seven months and two did not flower after ten months. The latter were given a cold treatment (5°C and short days for three months) and after this they flowered. All the hybrids lived for nine months and one hybrid was still alive and quite healthy after two years, showing the perennial habit of V. sicula.

The inflorescences of the hybrids was a raceme with only very occasionally two spikelets at the lower nodes, and were less branched than either V. membranacea or V. sicula (Plate 7). The culm was always erect as in V. sicula.

Table 14 shows measurements of characters of the inflorescence. The hybrids were intermediate in inflorescence characters except for pedicel length and lemma awn length, which were both smaller and similar to that of V. sicula, and the ovary tip was pubescent as in V. sicula. Pollen grains were smaller in the hybrids than in either parental species. Fig. 8 is a scatter diagram of inflorescence characters of parental species and hybrids and shows the intermediacy of the hybrids.

55 cells were observed at meiosis and there was a range of one to seven bivalents with a mean of 4.5 bivalents (Table 15, Plate 7). Fig. 9 shows the number of bivalents (including trivalents) and the mode was four bivalents. Two cells had a trivalent and the mean number of

Plate 7 a) Inflorescence of V. sicala (x 0.7)

b) Inflorescence of V. membranacea x V. sicala artificial
hybrid (x 1)

See Plate 5a for inflorescence of V. membranacea.

c) P.M.C. of V. membranacea x V. sicala with seven bivalents
at metaphase I (x 2,500)

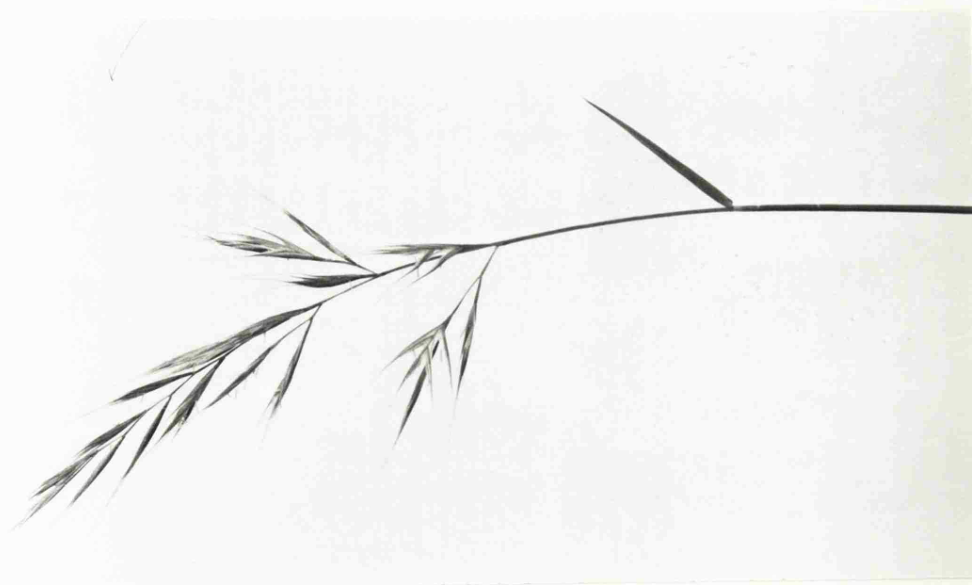
d) Stained pollen grains of V. sicala (x 400)

e) Crumpled and empty pollen grains of V. membranacea x
V. sicala (x 400)

Plate 7

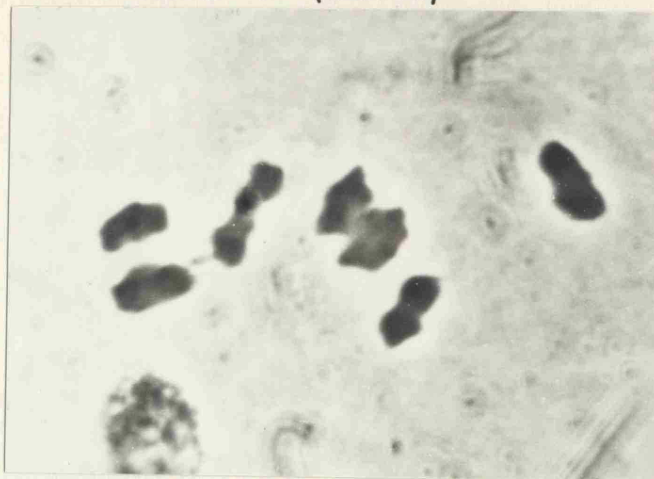


a

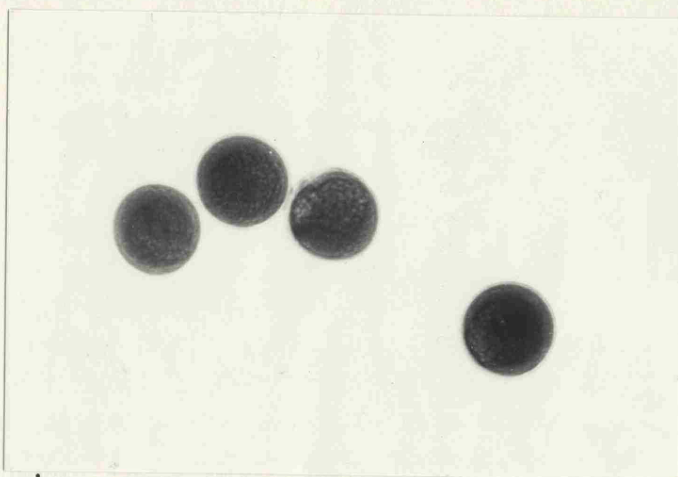


b

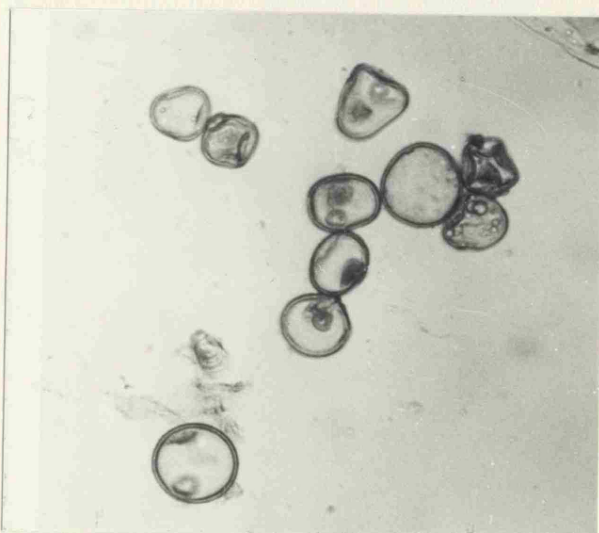
Plate 7 (cont.)



c



d



e

TABLE 14 Measurements of inflorescence characters for V. membranacea, V. sicula and V. membranacea x V. sicula hybrids

Inflorescence characters	V. membranacea	Hybrid	V. sicula
Lower glume length mm	0.5 - 1.2	0.6 - 1.5	0.5 - 2.1
Upper glume length (-awn) mm	6.7 - 10.6	6.4 - 6.8	5.1 - 7.0
Upper glume awn length mm	1.1 - 3.6	0 - 0.9	0
Lemna length (-awn) mm	6.7 - 10.6	5.4 - 6.6	4.4 - 6.1
Lemna awn length mm	6.2 - 13.5	3.4 - 5.0	2.0 - 3.3
Glume ratio	$< \frac{1}{10}$	$< \frac{1}{5}$	$\frac{1}{10} - \frac{1}{3}$
Anther length mm	0.4 - 0.7	1.2	1.9 - 2.8
Pedice length mm	2.3 - 5.4	1.2 - 1.6	1.0 - 2.0
Callus length mm	0.5 - 0.8	0.3 - 0.4	0.2
Ovary, glabrous or pubescent	G	P	P
Pollen grain size μ m	27 - 31	16 - 24	25 - 30
Chromosome number	14	14	14

FIGURE 8. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V. MEMBRANACEA, V. SICULA AND HYBRIDS.

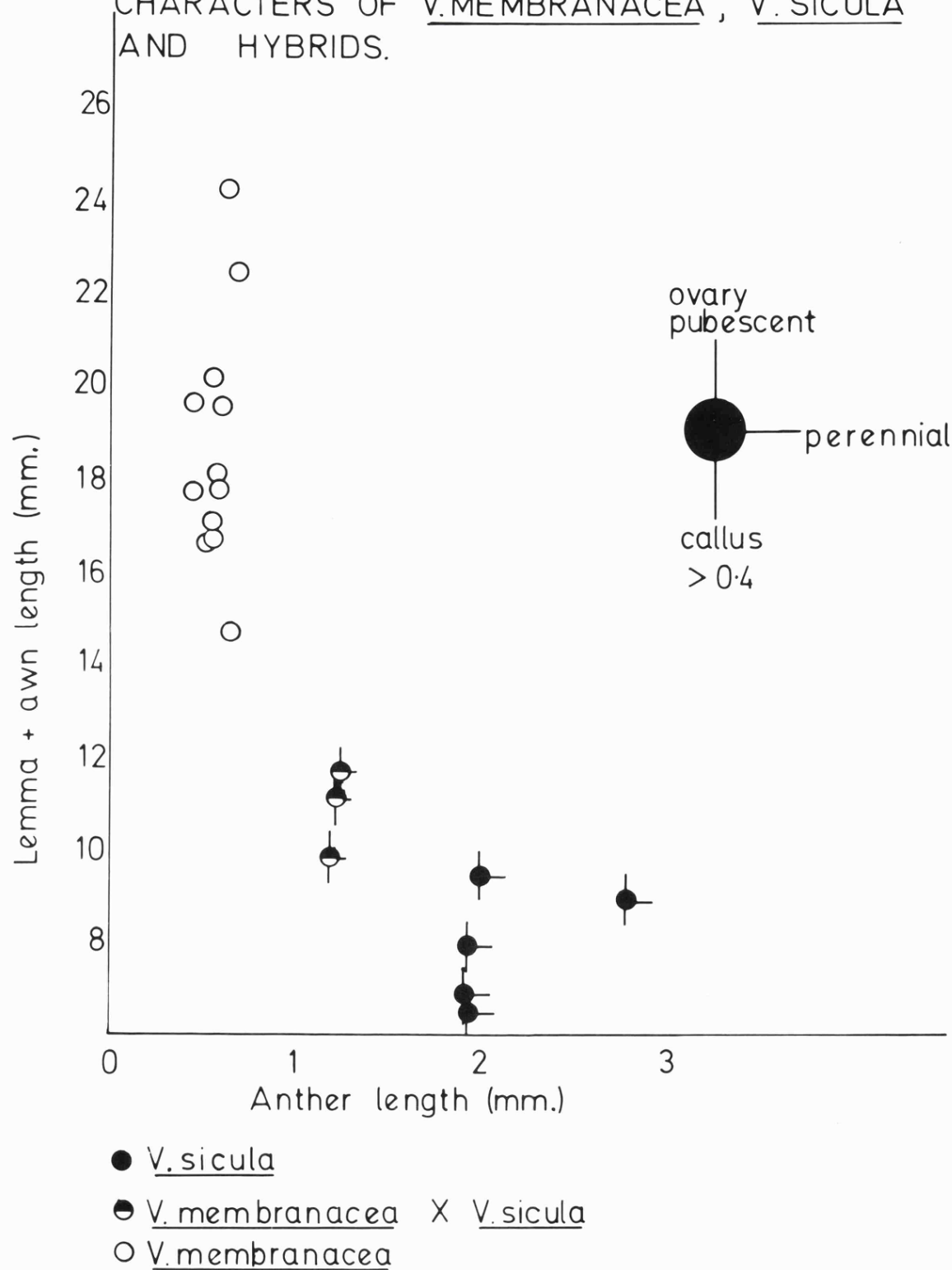
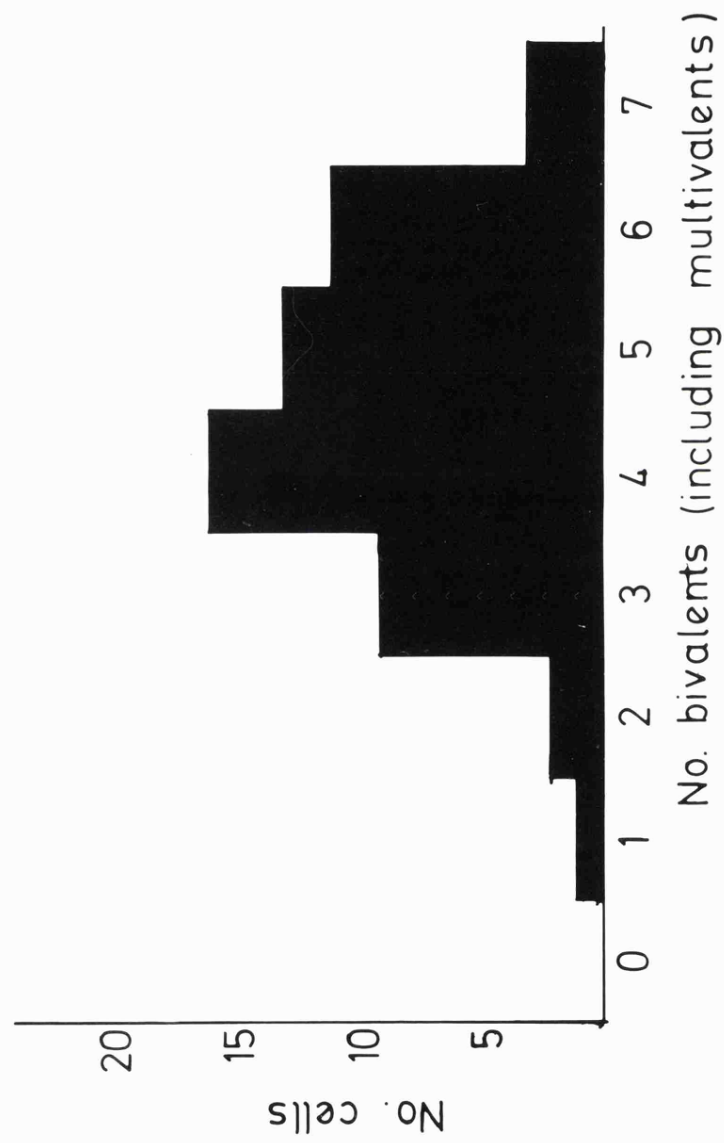


TABLE 15 Chromosome associations at meiosis in V. membranacea x
V. sicala hybrids

No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
1	7	0	3
	6	2	11
	5	4	13
	4	6	14
	3	5	2
	3	8	9
	2	10	2
	1	12	1

FIGURE 9. HISTOGRAM OF NUMBER OF BIVALENTS
(INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS
IN V. MEMBRANACEA X V. SICULA HYBRIDS.



chiasmata per cell was 7.95. The pollen grains often contained some cytoplasm but were not filled and were 100% sterile. The florets were opened during flowering and the anthers were exerted but were indehiscent and the hybrids were seed sterile. See No. 25 for reciprocal.

22. V. sicula x V. alopecuroides

419 florets were pollinated but no caryopses were produced. See No. 4 for reciprocal.

23. V. sicula x V. geniculata

548 florets were pollinated and 140 caryopses were produced. In 80 caryopses dissected during embryo culture the embryo could not be found. Of the remaining 60 caryopses which had embryos, five germinated and one mature hybrid plant was obtained. The other four seedlings died when transferred to soil, probably as a result of insufficient root growth in the agar medium.

The one hybrid grew vigorously and flowered after three months. The hybrid plant was intermediate in general appearance between V. geniculata and V. sicula. The inflorescence was branched but not so spreading as in V. geniculata (Plate 8). The hybrid resembled V. geniculata more closely for lower glume length, but was closer to V. sicula for pedicel length, and the anther length was smaller than in either parental species. The habit of this hybrid is unknown as it was taken for a pressed specimen. Inflorescences were collected for meiotic study but a suitable stage of meiosis was not found. The florets opened and the anthers were exerted during flowering but they were indehiscent. The pollen was 100% sterile and the hybrid plant was seed sterile. See No. 13 for reciprocal.

Plate 8 a) Inflorescence of V. geniculata (x 0.6)

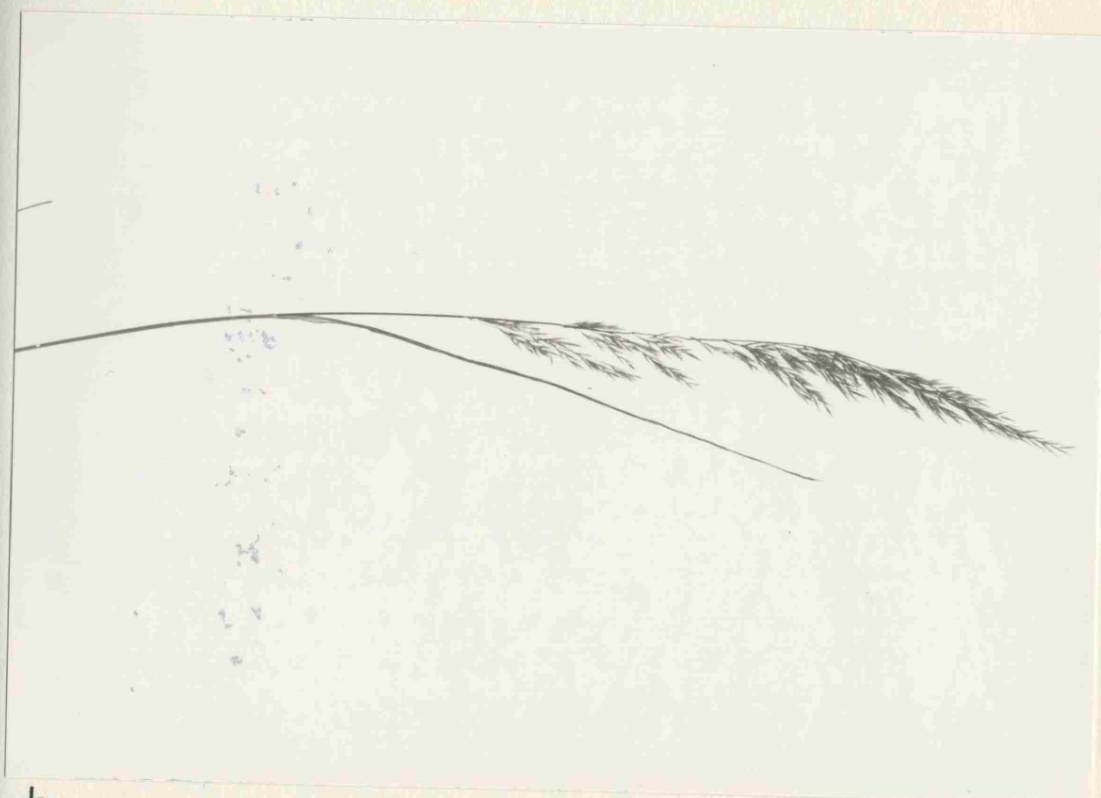
b) Inflorescence of V. sicula x V. geniculata artificial
hybrid (x 0.7)

See Plate 7a for inflorescence of V. sicula

Plate 8



a



b

TABLE 16 Measurements of inflorescence characters for V. sicula, V. geniculata and
V. sicula x V. geniculata hybrid

Inflorescence characters	<u>V. sicula</u>	Hybrid*	<u>V. geniculata</u>
Lower glume length mm	0.5 - 2.1	3.7 (3.2 - 4.2)	2.3 - 4.5
Upper glume length (-awn) mm	5.1 - 7.0	5.7 (5.4 - 6.2)	5.4 - 8.1
Upper glume awn length mm	0	0	0 - 0.4
Lemna length (-awn) mm	4.4 - 6.1	4.9 (4.6 - 5.1)	4.3 - 7.4
Lemna awn length mm	2.0 - 3.3	2.9 (2.0 - 3.5)	2.6 - 4.5
Glume ratio	$\frac{1}{10} - \frac{1}{3}$	$\frac{3}{5}c$	$\frac{2}{5} - \frac{3}{5}$
Anther length mm	1.9 - 2.8	1.3 (1.0 - 1.8)	1.9 - 2.7
Pedicel length mm	1.0 - 2.0	1.8 (1.0 - 3.0)	4.1 - 7.6
Callus length mm	0.2	0.2	0.2
Ovary, glabrous or pubescent	P	P	P
Pollen grain size μm	25 - 30	27	26 - 29
Chromosome number	14	14	14

*The means and ranges, in brackets, are given for one hybrid plant.

24. V. sicula x V. ligustica

287 florets were pollinated but no caryopses were produced. See No. 17 for reciprocal.

25. V. sicula x V. membranacea

84 florets were pollinated but no caryopses were produced. See No. 21 for reciprocal.

Diploid-tetraploid combinations1. V. alopecuroides x V. fasciculata

109 florets were pollinated but no caryopses were produced. See No. 2 for reciprocal.

2. V. fasciculata x V. alopecuroides

138 florets were pollinated and two caryopses were produced. Of these, one was later found to be a self. The embryo from the other caryopsis germinated and grew to a mature plant.

The hybrid plant flowered after three months and produced many inflorescences. The hybrid was intermediate in general appearance between the parental species (Plate 9). V. fasciculata always has glabrous inflorescence parts whereas V. alopecuroides has pubescent lemmas; on some plants this pubescence is confined to long hairs on the lemma edges, whereas on others the outer lemma surface is also pubescent. The parental genotype had the latter type of pubescence, whereas the hybrid was pubescent only along the edges of the lemma. The hybrid inflorescences turned purple when mature, a character which is not found in the parental species.

Plate 9 a) Inflorescence of V. alopecuroides (x 0.8)

b) Inflorescence of V. fasciculata x V. alopecuroides artificial
hybrid (x 0.6)

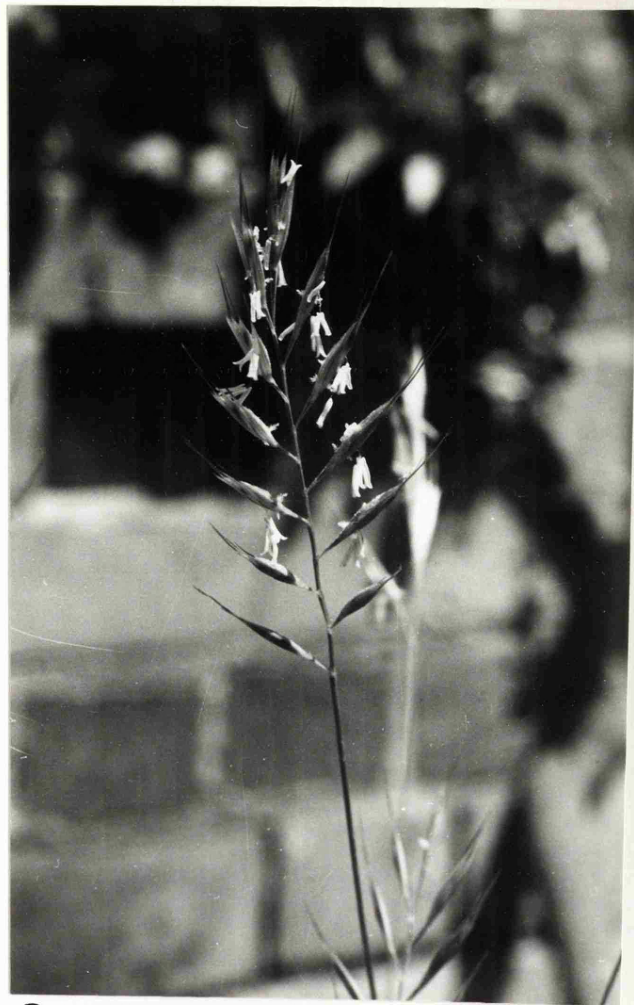
See Plate 10a for inflorescence of V. fasciculata

c) Scanning electron micrograph (S.E.M.) of the glabrous
ovary tip of V. alopecuroides (x 100)

d) S.E.M. of pubescent ovary tip of V. fasciculata (x 100)

e) S.E.M. of pubescent ovary tip of V. fasciculata x
V. alopecuroides (x 200)

Plate 9



a



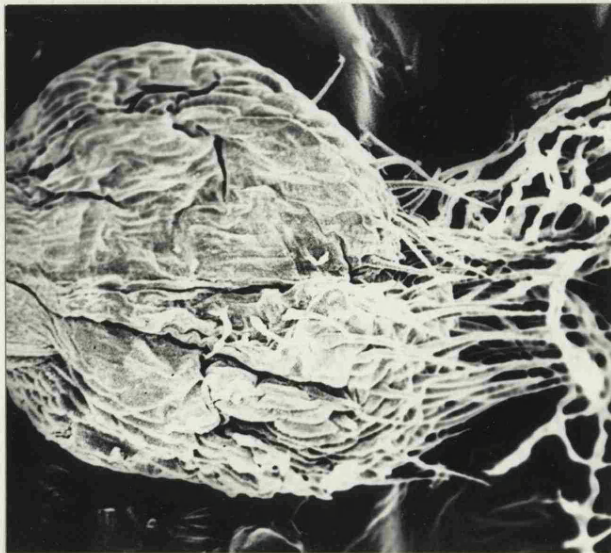
b

Plate 9 (cont.)

c



d



e



TABLE 17 Measurements of inflorescence characters for V. fasciculata, V. alopecuroides and
V. fasciculata x V. alopecuroides

Inflorescence characters	V. fasciculata	Hybrid*	V. alopecuroides
Lower glume length mm	0.3 - 0.8	0.8 (0.5 - 1.2)	1.1 - 2.1
Upper glume length (-awn) mm	9.5 - 16.1	16.0 (13.0 - 19.0)	10.2 - 13.5
Upper glume awn length mm	1.7 - 11.1	7.8 (6.0 - 9.0)	0.4 - 1.2
Lemma length (-awn) mm	8.9 - 16.0	14.3 (12.5 - 17.0)	8.5 - 11.6
Lemma awn length mm	9.8 - 16.4	13.1 (11.0 - 16.5)	5.4 - 6.7
Glume ratio	$\frac{1}{12}$	$\frac{1}{12}$	$\frac{1}{6}$
Anther length mm	0.6 - 1.1	1.5 (1.2 - 1.8)	2.4 - 3.0
Pedicele length mm	4.0 - 5.6	6.0 (5.5 - 6.7)	2.5 - 3.8
Callus length mm	0.5 - 0.8	(0.5 - 0.8)	0.5 - 0.8
Ovary, glabrous or pubescent	P	P	G
Chromosome number	28	21	14

*The means and ranges, in brackets, are given for one hybrid plant.

TABLE 18 Chromosome associations at meiosis in *V. fasciculata* x
V. alopecuroides hybrid

No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
	9	3	1
	8	5	1
	7	7	4
	6	9	3
1	5	7	1
	5	11	3
2	3	9	1
	4	13	3
3	0	15	1
	3	15	1

FIGURE 10. HISTOGRAM OF NUMBER OF BIVALENTS
(INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN
V. FASCICULATA X V. ALOPECUROS.

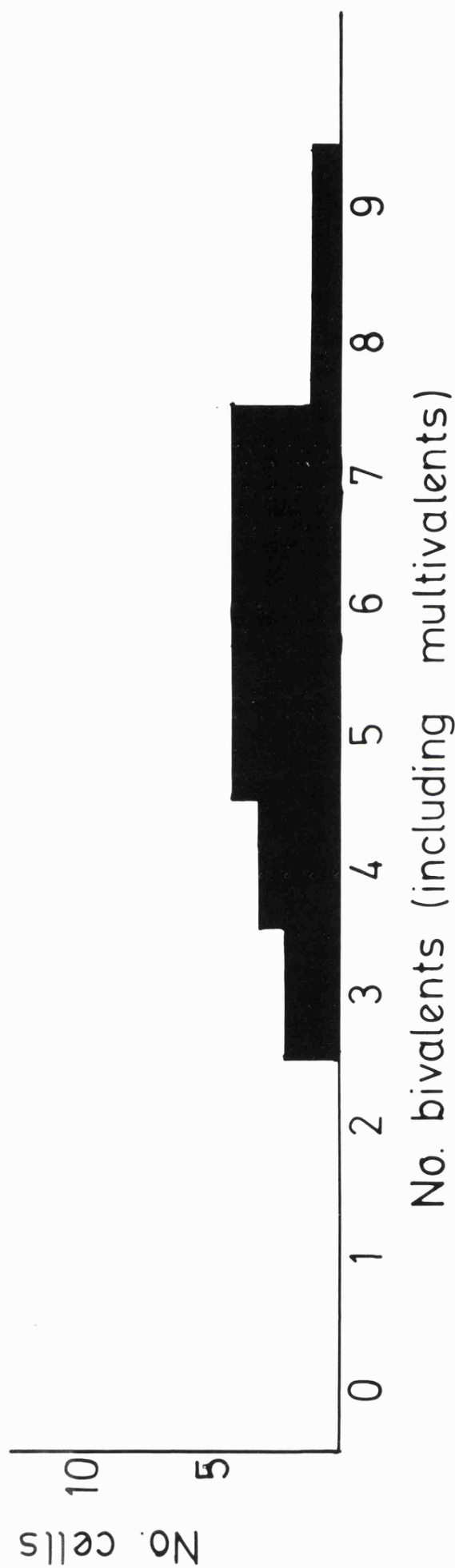


Table 17 shows the measurements of inflorescence characters for the parental species and the hybrid. The hybrid was intermediate for anther length but for upper glume length (-awn), upper glume awn length, lemma length (-awn) and lemma awn length and ovary tip pubescence the hybrid was more similar to V. fasciculata (Plate 9).

19 cells were observed at meiosis. There was a range of three to nine bivalents, the mean number of bivalents was 5.3, and three cells had trivalents (Table 18). Fig. 10 shows the number of bivalents (including trivalents). 100% of the pollen was sterile and the anthers, which were exerted at anthesis, were indehiscent. The hybrid was seed sterile. See No. 1 for reciprocal.

3. V. fasciculata x V. geniculata

116 florets were pollinated and 15 caryopses were produced. Embryos were found in 13 of the latter and of these, four embryos germinated and grew to mature plants.

The hybrids were vigorous and flowered about two months after germination. The inflorescence was branched and intermediate in general appearance between that of the parental species (Plate 10).

Measurements of inflorescence characters for parental species and hybrids are given in Table 19. The inflorescence characters in the hybrids were intermediate between those of the parental species except for the following:

Upper glume length (-awn) and lemma awn length were closer to those of V. fasciculata and upper glume awn length ~~was~~ closer to that of V. geniculata. The pollen grains were smaller than those of either parental species. Fig. 11 is a scatter diagram of inflorescence characters for parental species and hybrids and shows the closeness

Plate 10 a) Inflorescence of V. fasciculata (x 0.8)

b) Inflorescence of V. fasciculata x V. geniculata artificial
hybrid (x 0.6)

See Plate 8a for inflorescence of V. geniculata

c) P.M.C. of V. fasciculata x V. geniculata with five bivalents
and eleven univalents at metaphase I (x 2,500)

d) & e) Diad and tetrad stage of P.M.C. meiosis in
V. fasciculata x V. geniculata (x 1,500)

Plate 10

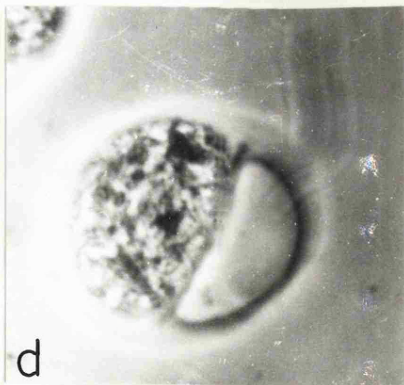
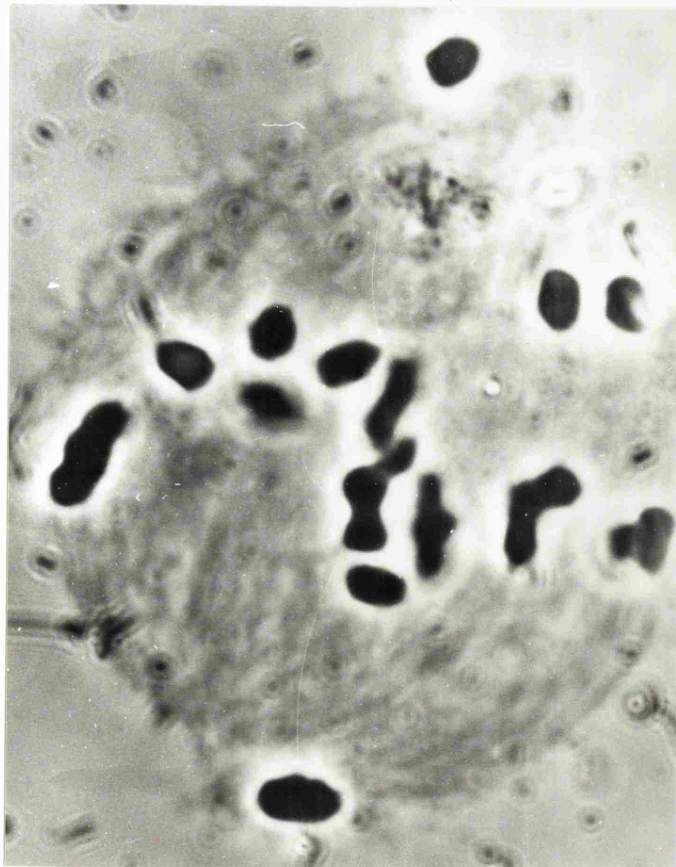
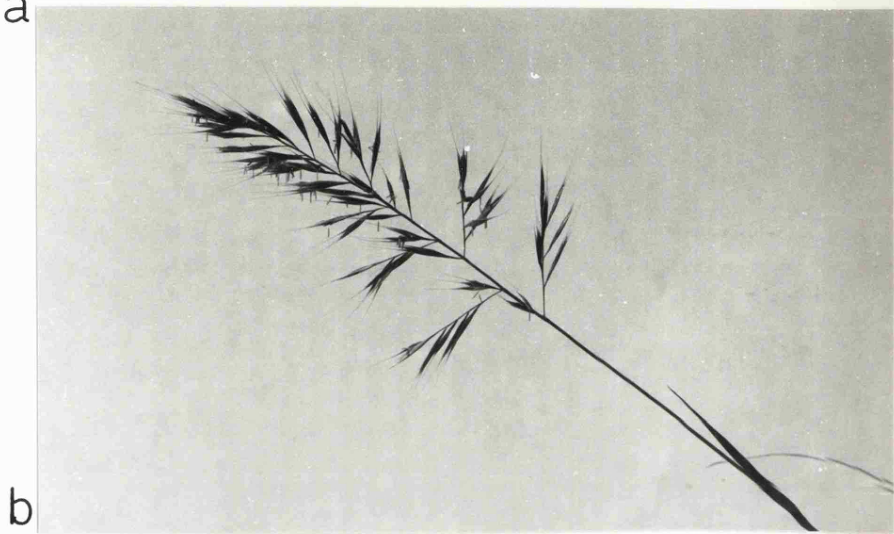
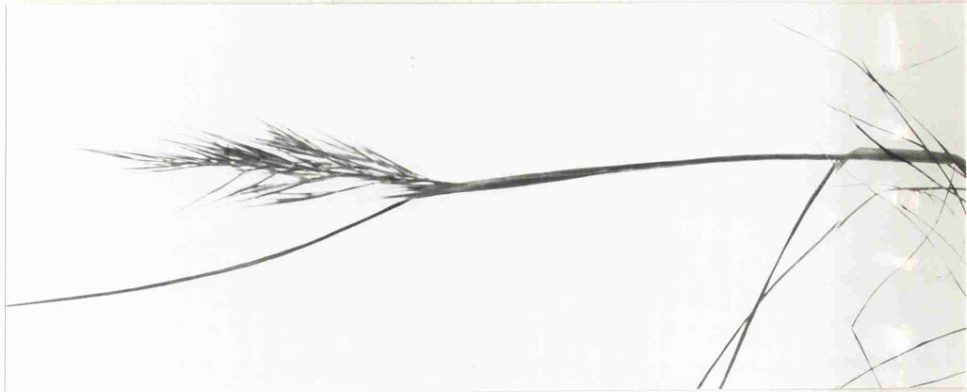
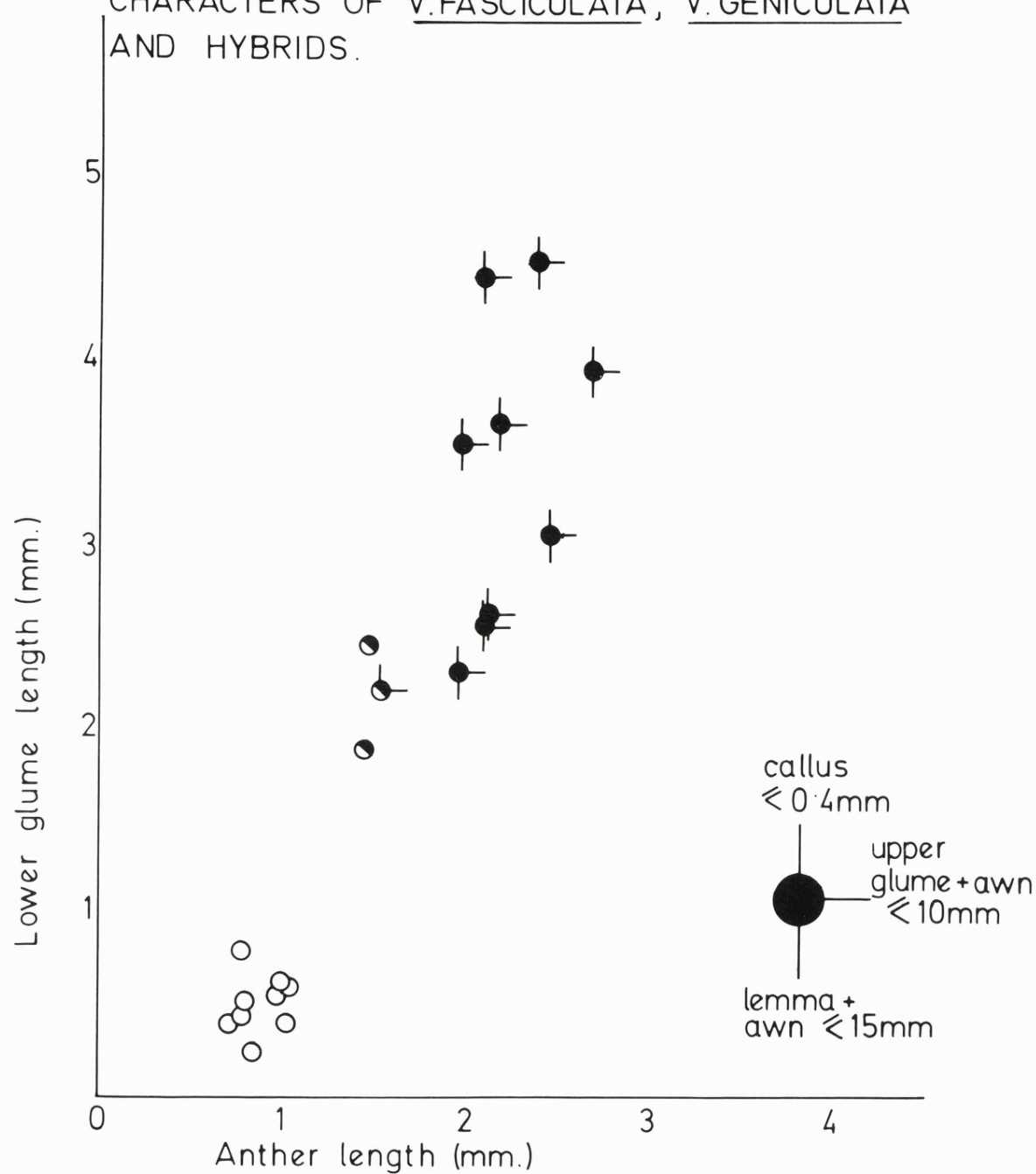


TABLE 19 Measurements of inflorescence characters for V. fasciculata, V. geniculata andV. fasciculata x V. geniculata hybrids

Inflorescence characters	<u>V. fasciculata</u>	Hybrid	<u>V. geniculata</u>
Lower glume length mm	0.3 - 0.8	1.9 - 2.5	2.3 - 4.5
Upper glume length (-awn) mm	9.5 - 16.1	9.6 - 12.9	5.4 - 8.1
Upper glume awn length mm	1.7 - 11.1	0	0 - 0.4
Lemna length (-awn) mm	8.9 - 16.0	7.8 - 9.7	4.3 - 7.4
Lemna awn length mm	9.8 - 16.4	8.9 - 13.1	2.6 - 4.5
Glume ratio	$< \frac{1}{12}$	$\frac{1}{10} - \frac{1}{5}$	$\frac{2}{5} - \frac{3}{5}$
Anther length mm	0.6 - 1.1	1.4 - 1.5	1.9 - 2.7
Pedicel length mm	4.0 - 5.6	5.4 - 6.3	4.1 - 7.6
Callus length mm	0.5 - 0.8	0.3 - 0.5	0.2
Ovary, glabrous or pubescent	P	P	P
Pollen grain size μ m	31 - 39	20 - 23	26 - 29
Chromosome number	28	21	14

FIGURE 11. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V.FASCICULATA, V.GENICULATA AND HYBRIDS.

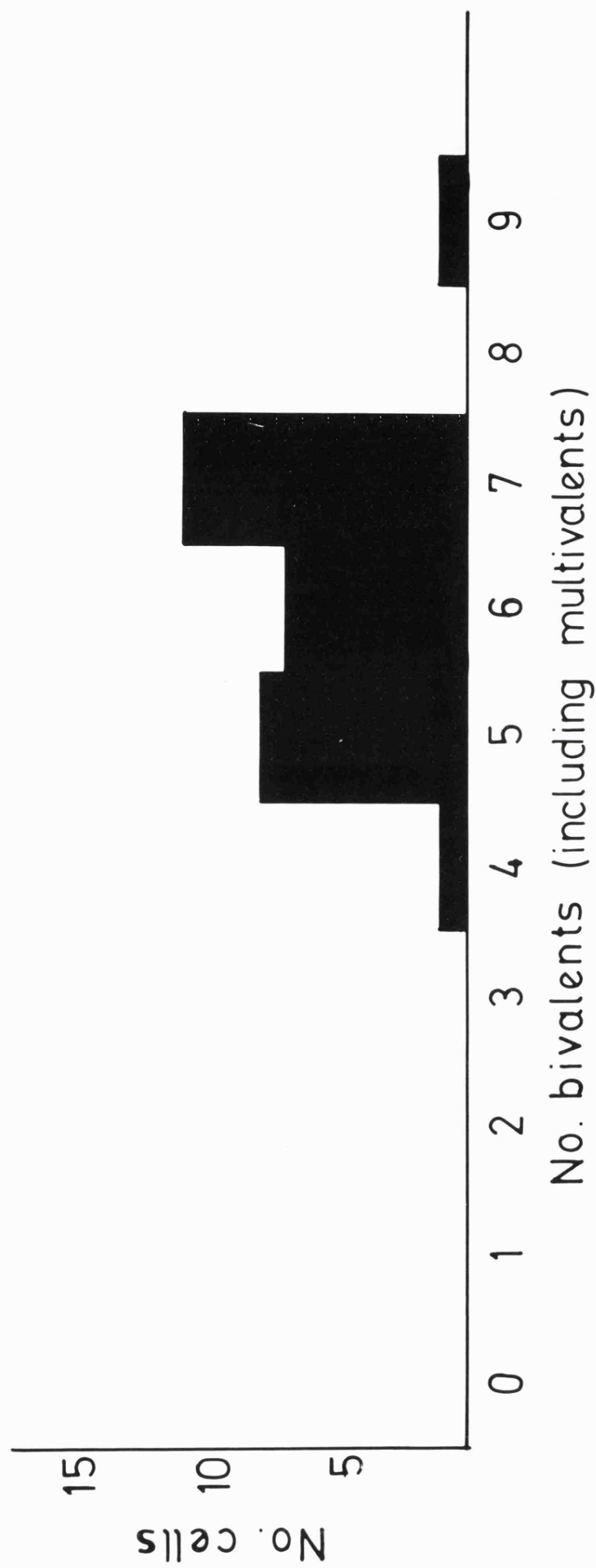


- V. geniculata
- ◐ V. fasciculata X V. geniculata hybrids
- V. fasciculata

TABLE 20 Chromosome associations at meiosis in V. fasciculata x
V. geniculata hybrids

No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
	9	3	1
	7	7	7
1	6	6	4
	6	9	5
3	3	13	1
1	5	8	1
	5	11	6
1	4	11	1
	4	13	1

FIGURE 12. HISTOGRAM OF NUMBER OF BIVALENTS
(INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN
V. FASCICULATA X V. GENICULATA HYBRIDS.



of the hybrids to the V. geniculata parent.

Observations on 27 cells at meiosis showed a range of four to nine bivalents, seven cells (26%) had one to three trivalents and one cell had a quinquivalent (Table 20, Plate 10). The mean bivalent number was 5.8. The mode number of bivalents (including trivalents) was seven (Fig. 12). The mean chiasmata number per cell was 8.57. The pollen was 100% sterile. The florets opened during flowering and the anthers were exerted, but they were indehiscent and the hybrids were seed sterile.

4. V. fasciculata x V. ligustica

100 florets were pollinated and 25 caryopses were produced. Embryos could only be found in 17 caryopses; of these ten embryos all germinated and grew to mature plants.

The hybrids were vigorous and flowered two months after germination and many inflorescences were produced. The inflorescences were well branched and more slender than in V. fasciculata (Plate 11). In glume length (-awn), glume awn length, lemma and lemma awn lengths the hybrids were similar to V. fasciculata. For anther length and callus length the hybrids were intermediate and the pollen grains in the hybrids were smaller than in either parental species (Table 21). Fig. 13 is a scatter diagram of inflorescence characters of parental species and the hybrids and shows the closeness of the hybrids to the V. fasciculata parent.

Observations on 103 cells at meiosis showed a range of three to nine bivalents and 15% of the cells had one or two trivalents (Table 22). The mean number of bivalents was 6.3. The mode number of bivalents (including trivalents) was 7 (Fig. 14). The mean number

Plate 11 a) Inflorescence of V. ligustica (x 0.8)

b) Inflorescence of V. fasciculata x V. ligustica artificial
hybrid (x 0.6)

See Plate 10a for inflorescence of V. fasciculata

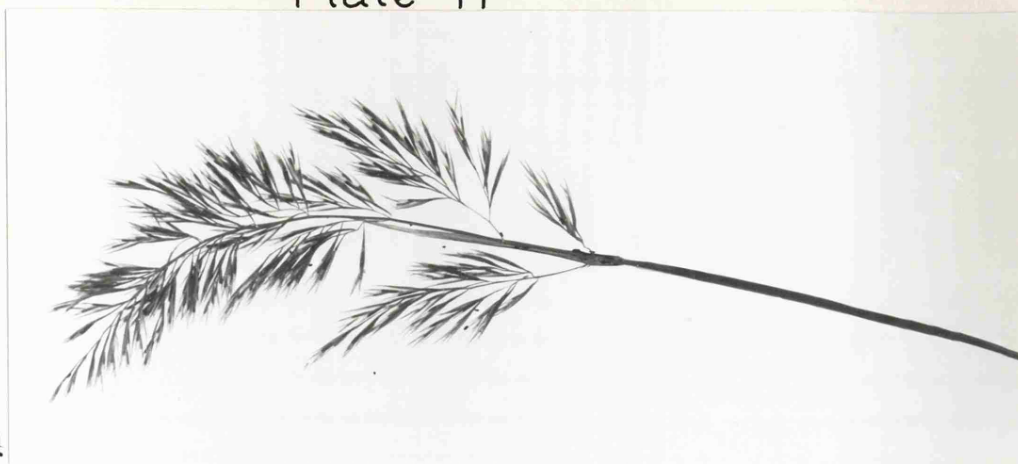
c) P.M.C. of V. fasciculata x V. ligustica with seven bivalents
and seven univalents at metaphase I (x 2,500)

d) Laggards at anaphase in V. fasciculata x V. ligustica P.M.C.
meiosis (x 1,800)

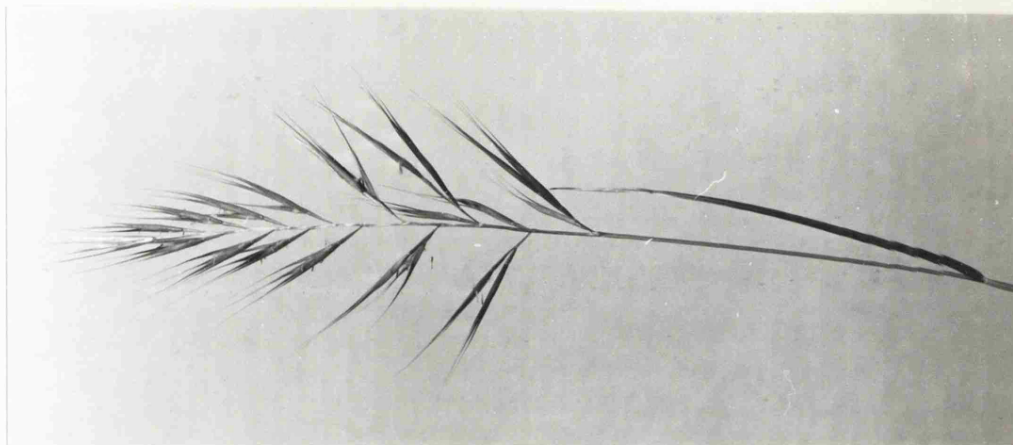
e) Micronuclei in tetrads in V. fasciculata x V. ligustica P.M.C.
meiosis (x 400)

Plate 11

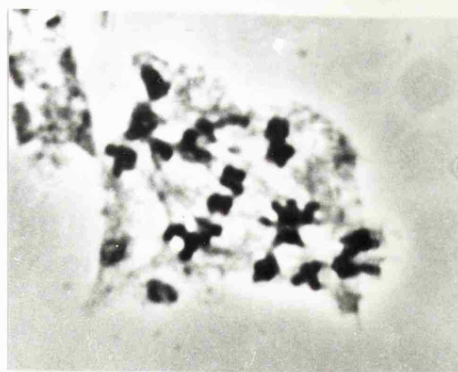
a



b



c



d

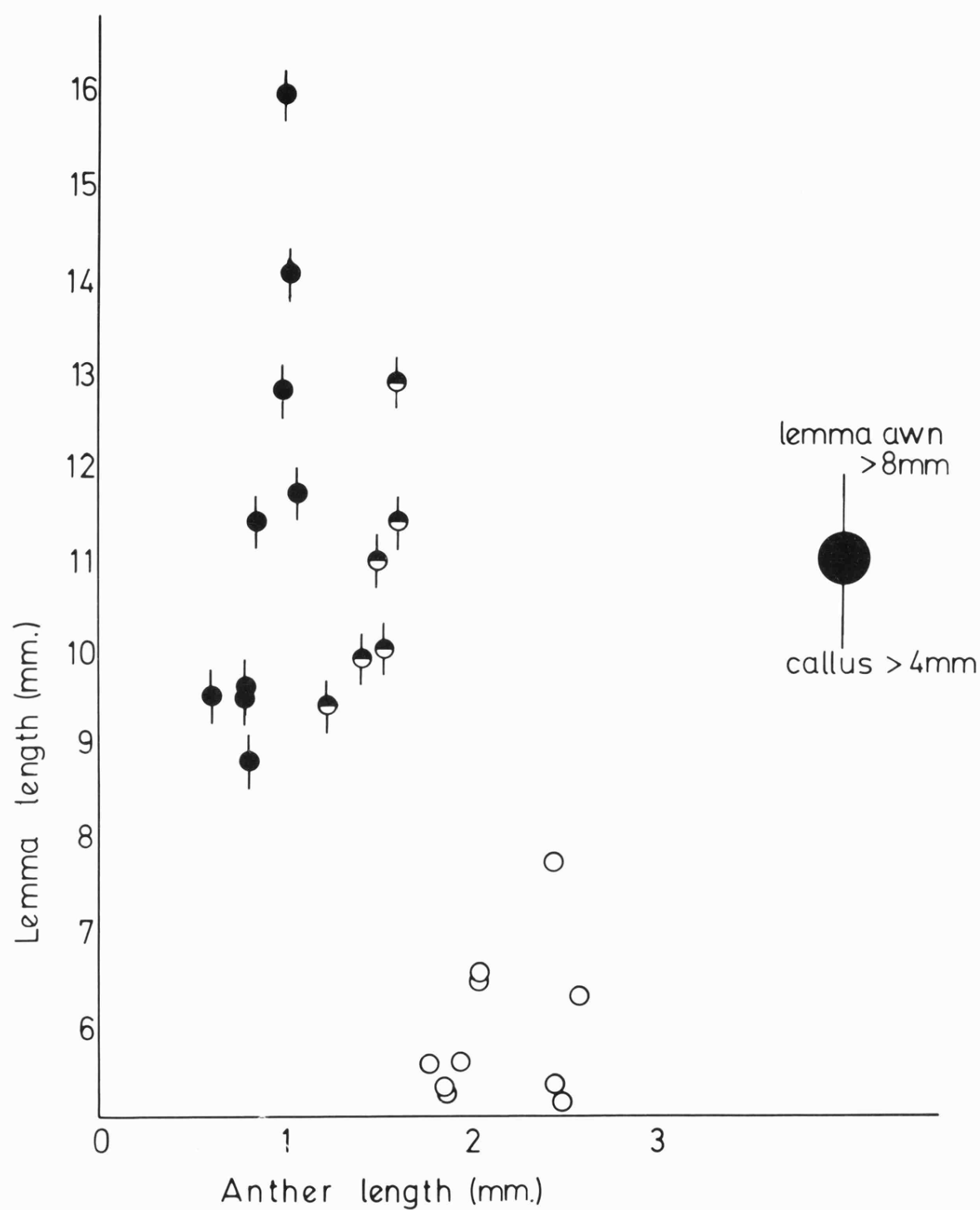


e

TABLE 21 Measurements of inflorescence characters for V. fasciculata, V. ligustica andV. fasciculata x V. ligustica hybrids

Inflorescence characters	<u>V. fasciculata</u>	Hybrid	<u>V. ligustica</u>
Lower glume length mm	0.3 - 0.8	0.3 - 0.7	0.2 - 0.7
Upper glume length (-awn) mm	9.5 - 16.1	12.2 - 13.6	6.6 - 10.8
Upper glume awn length mm	1.7 - 11.1	5.5 - 7.7	0 - 2.7
Lemma length (-awn) mm	8.9 - 16.0	9.4 - 12.7	5.4 - 7.8
Lemma awn length mm	9.8 - 16.4	10.0 - 16.0	2.7 - 6.6
Glume ratio	$< \frac{1}{12}$	$< \frac{1}{12}$	$< \frac{1}{10}$
Anther length mm	0.6 - 1.1	1.4 - 1.6	1.9 - 2.6
Pedicel length mm	4.0 - 5.6	4.6 - 5.6	3.1 - 5.1
Callus length mm	0.5 - 0.8	0.3 - 0.7	0.2
Ovary, pubescent or glabrous	P	P	P
Pollen grain size μ m	31 - 39	21 - 23	27 - 29
Chromosome number	28	21	14

FIGURE 13. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V. FASCICULATA, V. LIGUSTICA AND HYBRIDS.

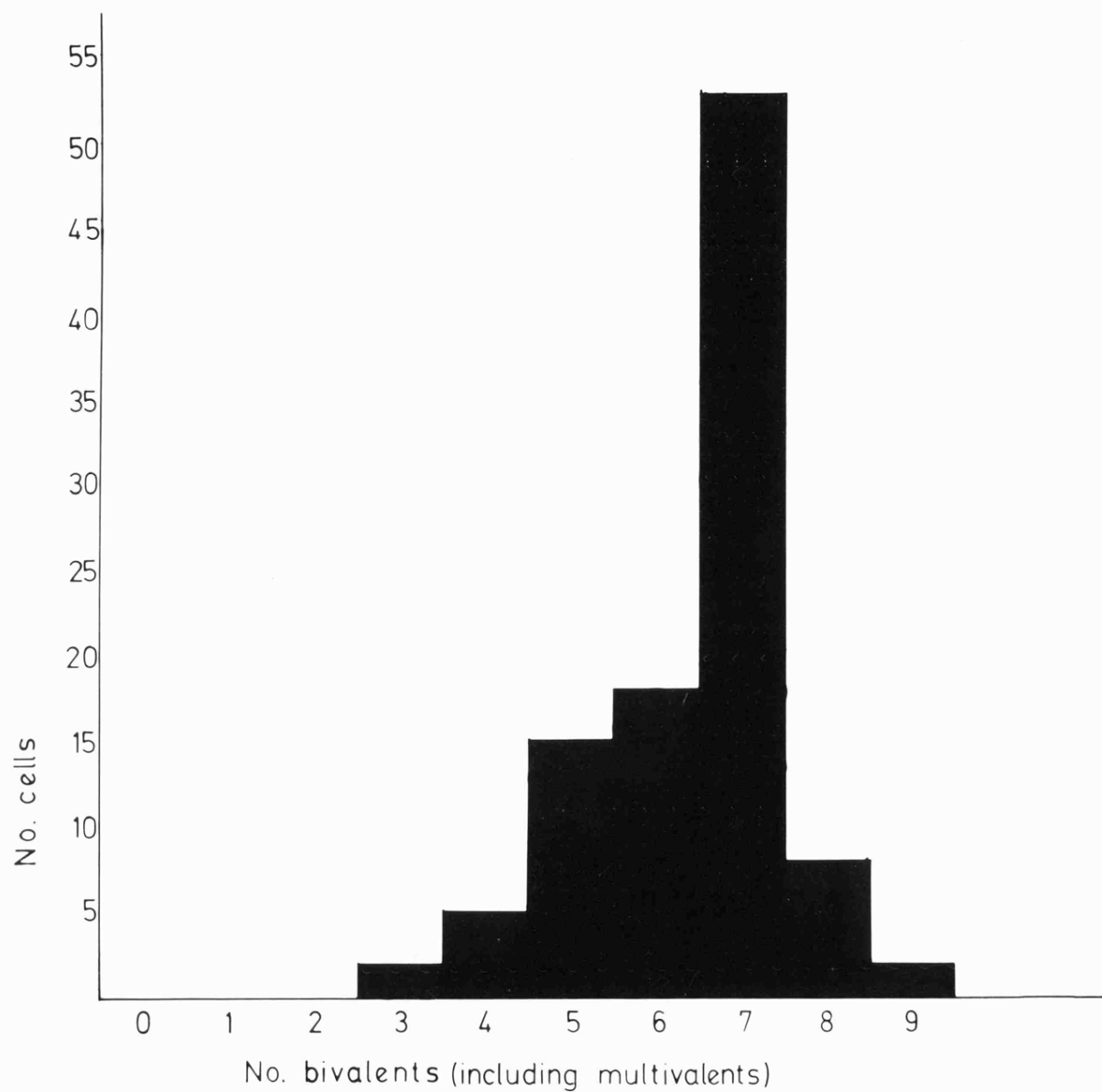


- V. fasciculata
- ◐ V. fasciculata X V. ligustica hybrids
- V. ligustica

TABLE 22 Chromosome associations at meiosis in *V. fasciculata* x
V. ligustica hybrids

No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
	9	3	2
	8	5	7
1	7	5	1
	7	7	45
2	5	5	2
1	6	6	6
	6	9	14
1	5	9	1
1	5	8	2
1	5	6	1
	5	11	11
1	4	11	1
1	4	10	1
1	4	9	1
1	4	8	1
	4	13	5
	3	15	2

FIGURE 14. HISTOGRAM OF NUMBER OF BIVALENTS (INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN V. FASCICULATA X V. LIGUSTICA HYBRIDS.



of chiasmata per cell was 7.78. Laggards and micronuclei were often seen at the later stages of meiosis (Plate 11). The pollen was 100% sterile. The florets opened during flowering and the anthers were exerted, but they were indehiscent and the hybrids were sterile.

5. V. fasciculata x V. membranacea

23 florets were pollinated and four caryopses were produced, one of which was later found to be a self. Of the three hybrid caryopses only one embryo germinated and this grew to a mature plant. This plant was allowed to flower and the inflorescences to mature to see if any F_2 caryopses were produced, but the hybrid was seed sterile and no pressed specimen was taken.

Only three cells were studied at meiosis; four to six bivalents (mostly rod bivalents) and one trivalent was observed. See No. 7 for reciprocal.

6. V. fasciculata x V. sicula

33 florets were pollinated and five caryopses were harvested. From these three mature hybrid plants were grown.

The hybrids took five months to flower and only a few inflorescences were produced. The plants were weak and died quickly after initiation of flowering, thus resembling V. fasciculata in the annual habit. The inflorescence was a raceme with sometimes two or more spikelets at the lower nodes (Plate 12). Two hybrids (numbers 105 and 108) had pubescent lemmas, pedicels, rhachilla segments and upper glumes and the third hybrid (number 55) had glabrous floral parts.

Lemmas of V. fasciculata are glabrous or minutely scabrid, whereas lemmas and upper glumes of V. sicula are either glabrous or pubescent. The pubescence of the individual V. sicula parents of these hybrids

Plate 12 a) Inflorescence of V. sicala (x 0.7)

b) Inflorescence of V. fasciculata x V. sicala artificial
hybrid (x 0.5)

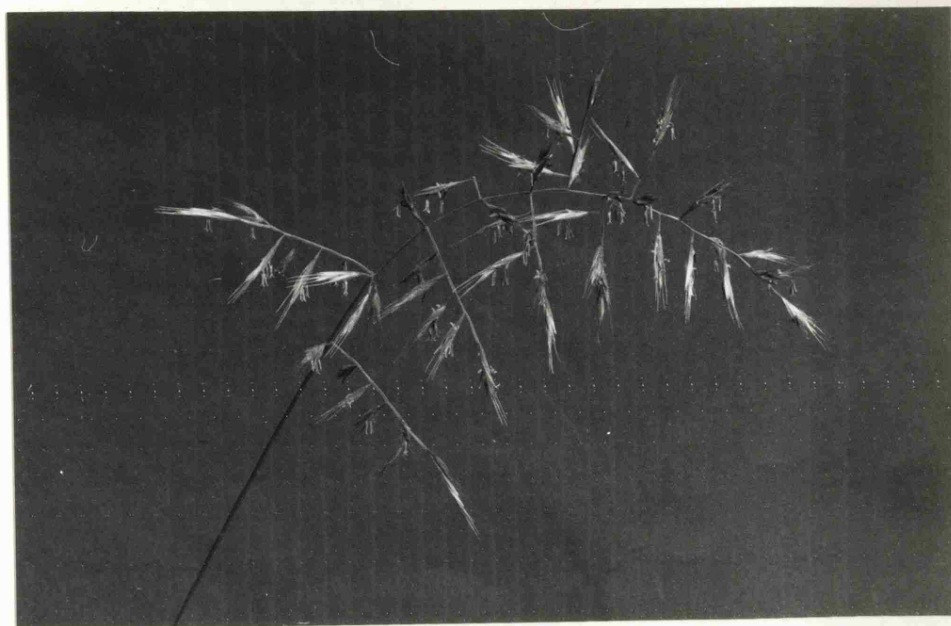
See Plate 10a for inflorescence of V. fasciculata

c) Root tip mitosis in V. fasciculata x V. sicala ($2n = 21$)
(x 2,600)

d) P.M.C. of V. fasciculata x V. sicala with seven bivalents and
seven univalents at metaphase I (x 2,500)

e) Diad stage of meiosis in V. fasciculata x V. sicala (x 1,500)

Plate 12

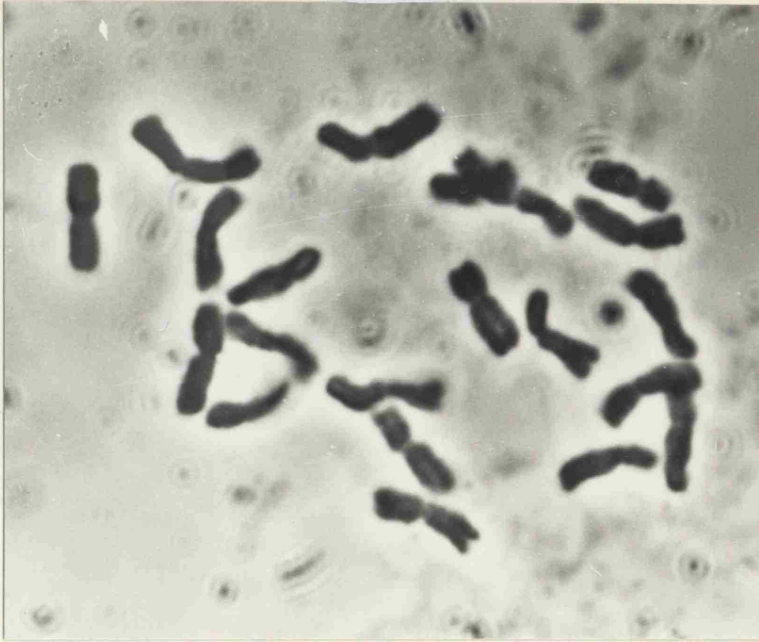


a

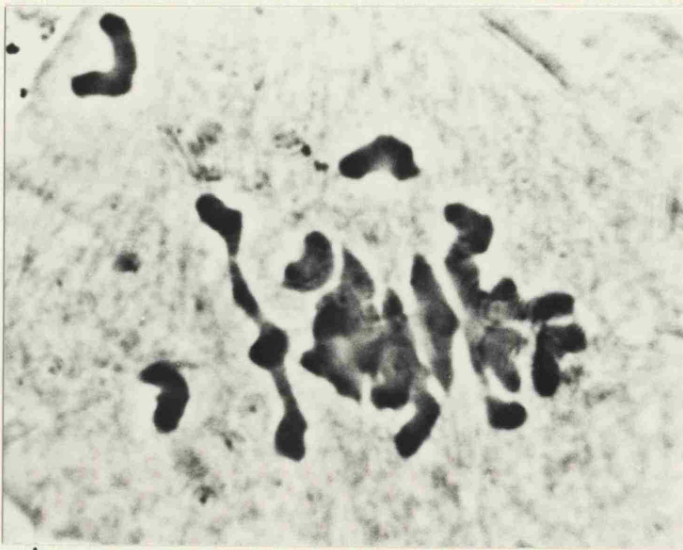


b

Plate 12 (cont.)



c



d



e

TABLE 23 Measurements of inflorescence characters for V. fasciculata, V. sicula and V. fasciculata x V. sicula hybrids

Inflorescence characters	<u>V. fasciculata</u>	Hybrid	<u>V. sicula</u>
Lower glume length mm	0.3 - 0.8	0.4 - 0.9	0.5 - 2.1
Upper glume length (-awn) mm	9.5 - 16.1	8.2 - 9.6	5.1 - 7.0
Upper glume awn length mm	1.7 - 11.1	0.6 - 1.3	0
Lemma length (-awn) mm	8.9 - 16.0	5.6 - 8.2	4.4 - 6.1
Lemma awn length mm	9.8 - 16.4	5.4 - 9.2	2.0 - 3.3
Glume ratio	$< \frac{1}{12}$	$< \frac{1}{10}$	$\frac{1}{10} - \frac{1}{3}$
Anther length mm	0.6 - 1.1	1.1 - 1.2	1.9 - 2.8
Pedicel length mm	4.0 - 5.6	1.5 - 2.7	1.0 - 2.0
Callus length mm	0.5 - 0.8	0.3 - 0.5	0.2
Ovary, pubescent or glabrous	P	P	P
Pollen grain size μ m	31 - 39	22 - 23	25 - 30
Chromosome number	28	21	14

FIGURE 15. SCATTER DIAGRAM OF INFLORESCENCE CHARACTERS OF V. FASCICULATA, V. SICULA AND HYBRIDS.

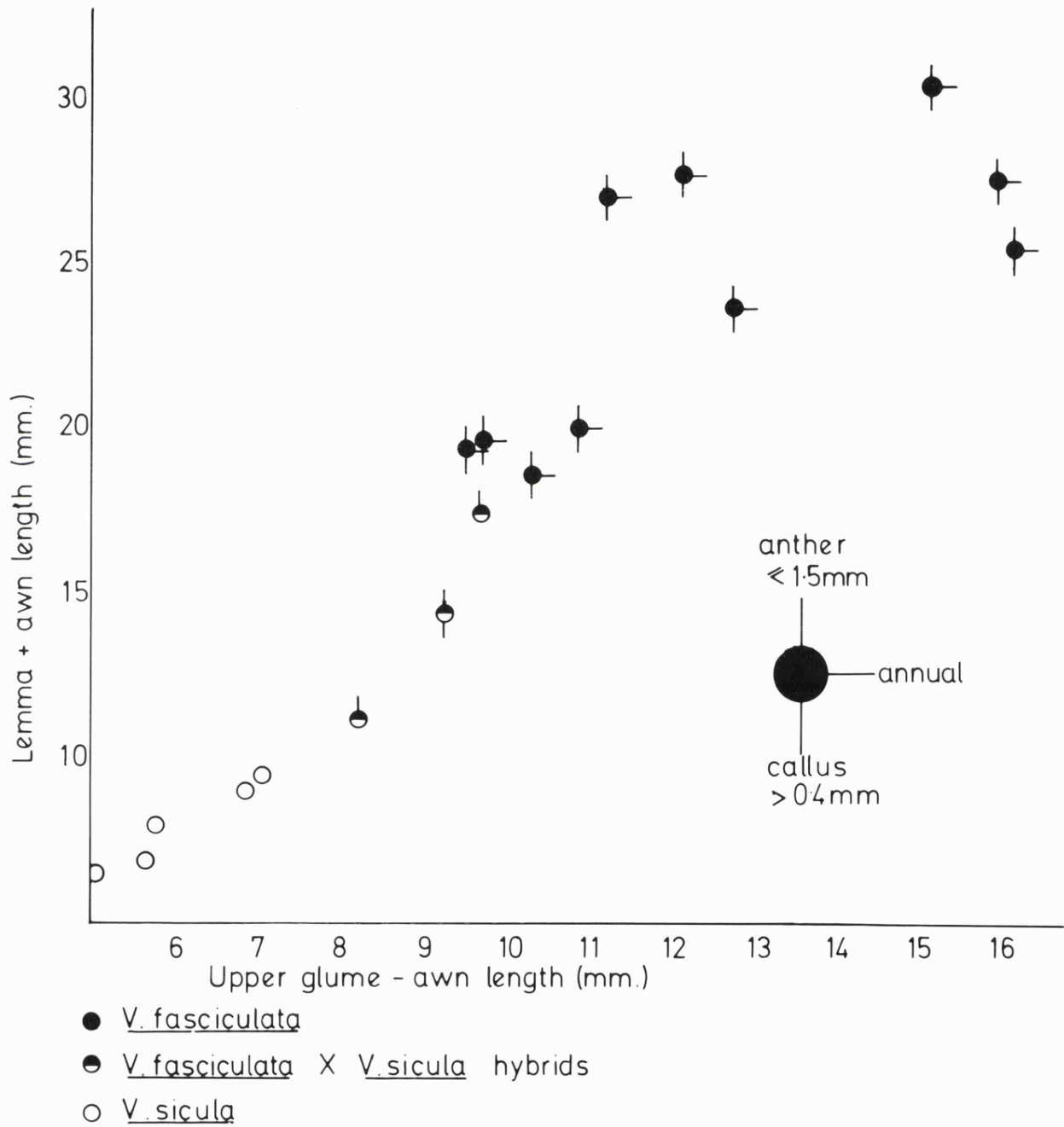
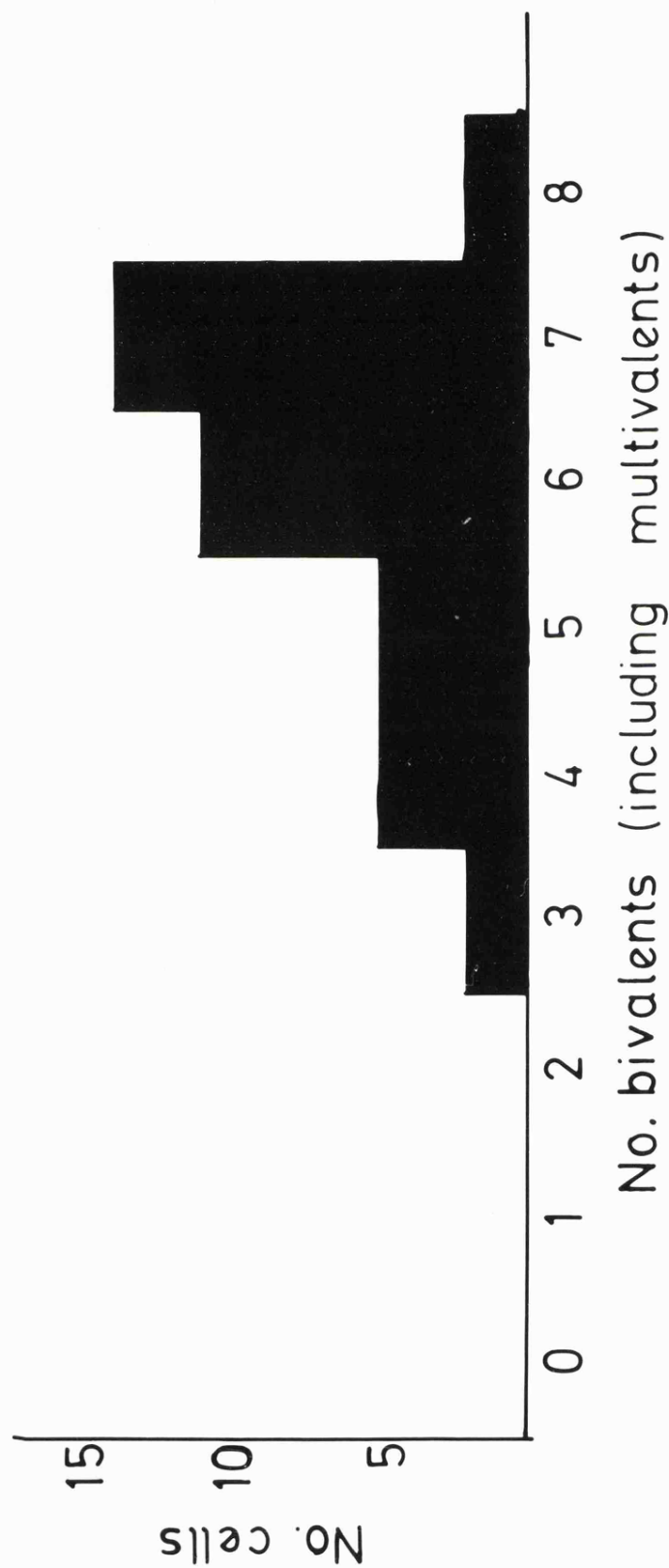


TABLE 24 Chromosome associations at meiosis in *V. fasciculata* x *V. sicula* hybrids

No. of quivalentents	No. of trivalents	No. of bivalents	No. of univalents	No. of cell observed
		8	5	1
	1	7	6	1
		7	7	12
	1	6	6	1
	1	6	5	1
		6	9	10
	1	5	9	1
		5	11	2
	2	3	11	1
	2	3	9	1
	1	4	10	1
		4	7	1
		4	13	3
	3	1	11	1
		3	15	2

FIGURE 16. HISTOGRAM OF NUMBER OF BIVALENTS (INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN V. FASCICULATA X V. SICULA HYBRIDS.



was unfortunately not recorded. Hybrids 105 and 108 had inflorescences only just exerted from the sheath, as in V. fasciculata, whereas hybrid 55 had the inflorescence well exerted from the sheath, as in V. sicula.

Measurements of parts of the inflorescence of parental species and hybrids are given in Table 23. The hybrids were intermediate for all inflorescence characters except for pedicel length, which was more similar to V. sicula. Pollen grains in the hybrids were smaller than in either parental species. Fig. 15 is a scatter diagram of inflorescence characters of parental species and the hybrids and shows the intermediacy of the hybrids.

Observations of 38 cells at meiosis showed that the number of bivalents ranged from one to eight and eight cells (21%) had one to three trivalents (Table 24, Plate 12). The mean bivalent number was 5.6. The mode number of bivalents (including trivalents) was seven (Fig. 16). The mean number of chiasmata per cell was 8.56. The pollen was 100% sterile. The florets opened during flowering, and the anthers were exerted but they were indehiscent and the hybrids were seed sterile.

7. V. membranacea x V. fasciculata

125 florets were pollinated and 27 caryopses were produced. Embryos were not found in ten caryopses but of the remaining 16 embryos, seven germinated and grew to mature plants.

The hybrids took three months to flower and the plants were vigorous and produced many inflorescences. The hybrids were intermediate in general appearance between the parental species, which are themselves superficially very similar (Plate 13). Measurements of inflorescence parts of parental species and hybrids are given in Table 25. The hybrids

Plate 13 a) Inflorescence of V. membranacea (x 1)

b) Inflorescence of V. membranacea x V. fasciculata artificial
hybrid (x 0.8)

See Plate 10a for inflorescence of V. fasciculata

c) Root tip mitosis in V. membranacea x V. fasciculata
($2n = 21$) (x 2,500)

d) P.M.C. of V. membranacea x V. fasciculata with five bivalents
and eleven univalents at metaphase I (x 2,500)

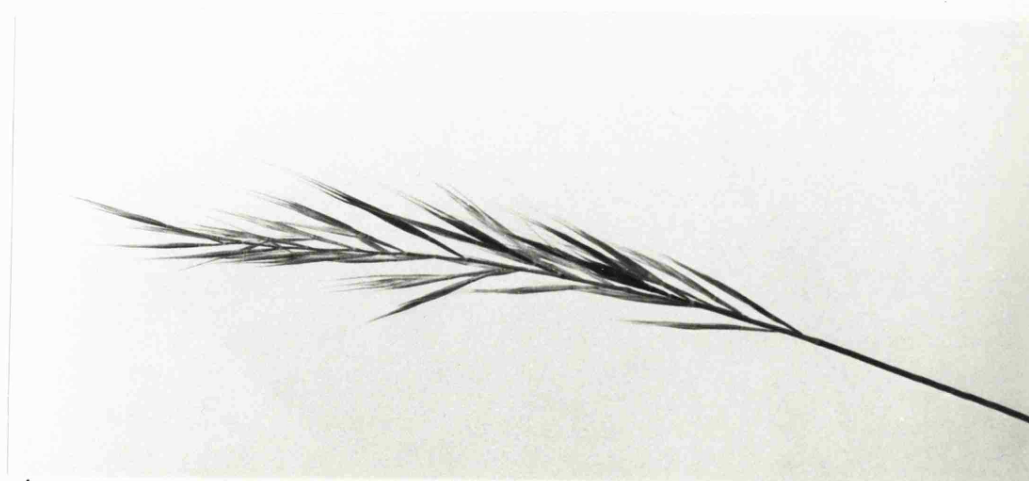
e) P.M.C. of V. membranacea x V. fasciculata with two trivalents,
three bivalents and nine univalents at metaphase I (x 2,500)

f) Abnormal tetrad formation in V. membranacea x V. fasciculata
P.M.C. meiosis (x 400)

Plate 13



a

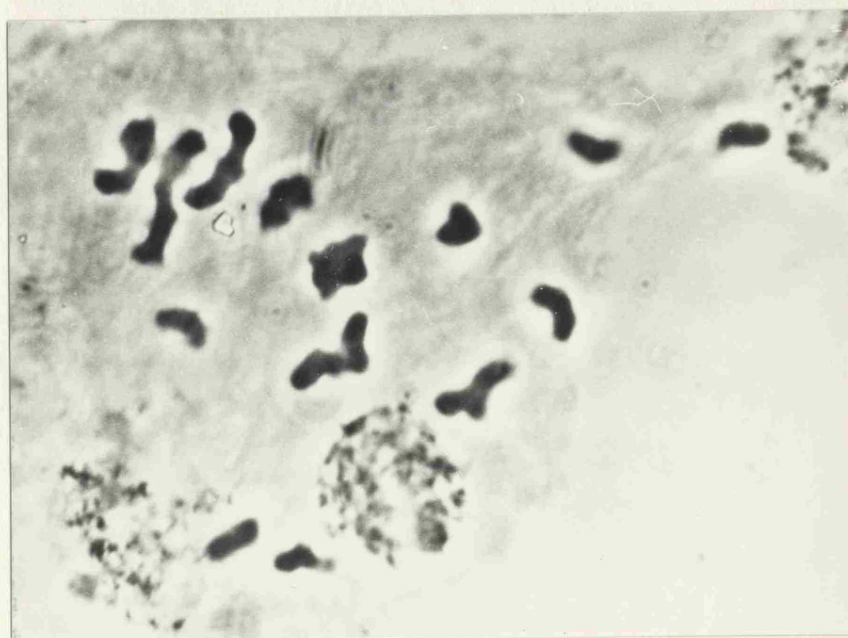


b

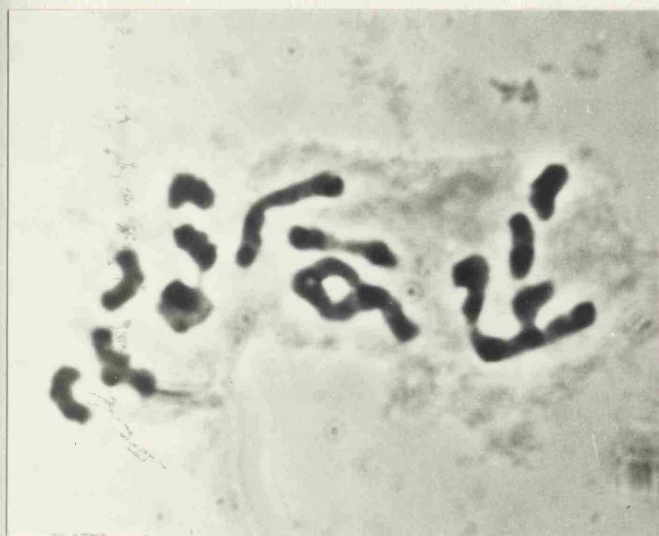
Plate 13 (cont.)



c



d



e



f

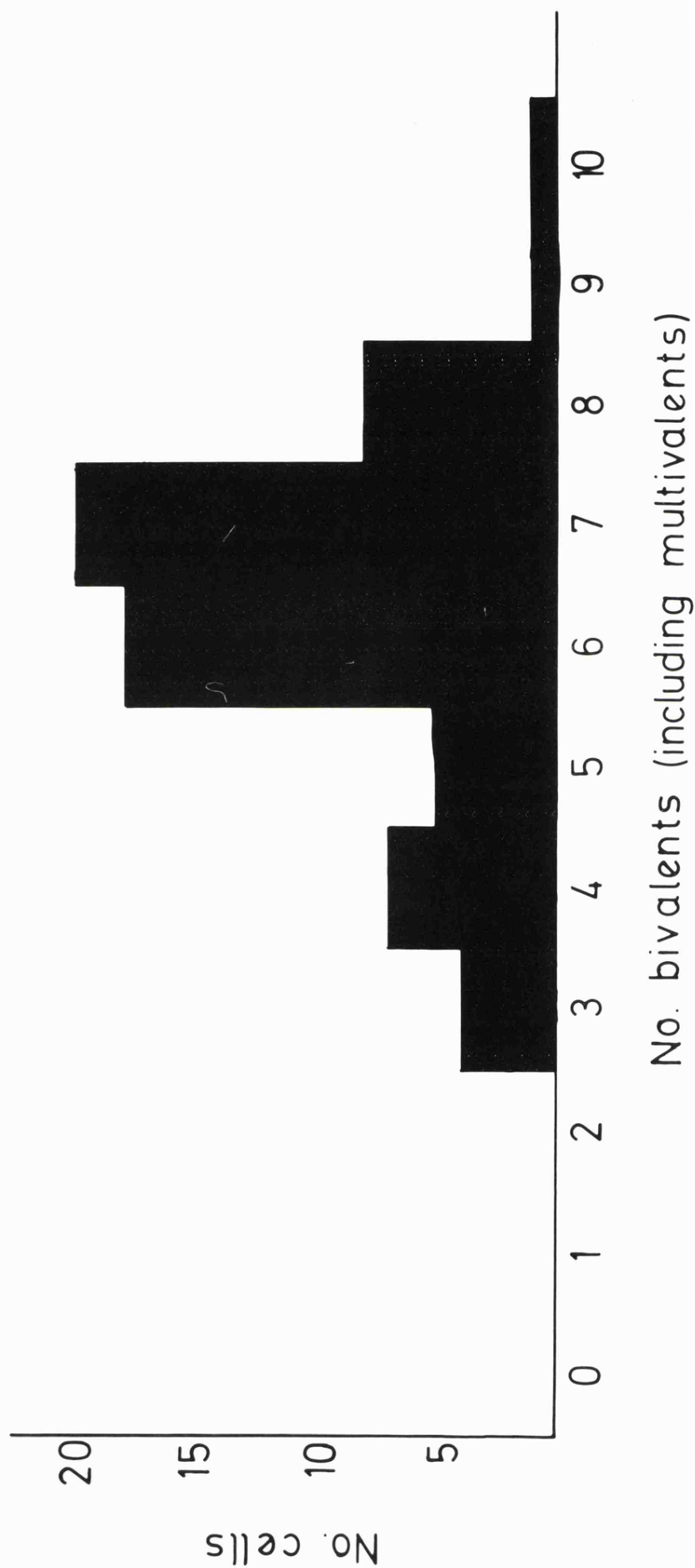
TABLE 25 Measurements of inflorescence characters for V. membranacea, V. fasciculata andV. membranacea x V. fasciculata hybrids

Inflorescence characters	<u>V. membranacea</u>	Hybrid	<u>V. fasciculata</u>
Lower glume length mm	0.5 - 1.2	0.2 - 0.8	0.3 - 0.8
Upper glume length (-awn) mm	6.7 - 10.6	10.0 - 12.1	9.5 - 16.1
Upper glume awn length mm	1.1 - 3.6	3.5 - 4.0	1.7 - 11.1
Lemma length (-awn) mm	6.7 - 10.6	8.9 - 13.8	8.9 - 16.0
Lemma awn length mm	6.2 - 13.5	9.7 - 15.4	9.8 - 16.4
Glume ratio	$< \frac{1}{10}$	$< \frac{1}{12}$	$< \frac{1}{12}$
Anther length mm	0.4 - 0.7	0.7 - 1.0	0.6 - 1.1
Pedicel length mm	2.3 - 5.4	3.8 - 5.9	4.0 - 5.6
Callus length mm	0.5 - 0.8	0.6 - 0.9	0.5 - 0.8
Ovary, pubescent or glabrous	G	P	P
Pollen grain size μ m	27 - 31	23 - 27	31 - 39
Chromosome number	14	21	28

TABLE 26 Chromosome associations at meiosis in V. membranacea x V. fasciculata hybrids.

No. of quivalentents	No. of quadrivalents	No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
1		1	9		1
			9	3	1
			8	5	4
		1	7	4	4
			7	7	9
	1		6	5	1
		3	4	4	1
		2	5	5	5
		1	6	6	4
			6	9	13
	1	3	2	3	1
		2	4	7	2
		1	5	8	2
			5	11	3
1		1	3	7	1
		1	4	10	1
			4	13	4
		2	2	11	1
		2	2	8	1
		1	3	13	1
			3	15	1
		2	1	11	3

FIGURE 17. HISTOGRAM OF NUMBER OF BIVALENTS
(INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN
V. MEMBRANACEA X V. FASCICULATA HYBRIDS.



were intermediate for all inflorescence characters except that the callus length was slightly greater than in either parental species. Pollen grains were smaller in the hybrids than in both the parental species. The hybrids had pubescent ovary tips as in V. fasciculata.

Observations on 64 cells at meiosis showed that the number of bivalents ranged from one to nine and 46% of the cells had one to three trivalents (Table 26, Plate 13). The mean bivalent number was 5.4. The number of bivalents (including multivalents) had a mode of seven (Fig. 17). The bivalents were mostly rod bivalents with one or two ring bivalents. Pollen grain sterility was 100% and in some anthers there were no pollen grains at all. The anthers were exerted during flowering but were indehiscent and the hybrids were seed sterile. See No. 5 for reciprocal.

b. Intergeneric Vulpia - Festuca crosses

Diploid-hexaploid combinations

1. F. rubra x V. sicula

354 florets were pollinated and 33 caryopses were produced. Two caryopses were later found to be selfs. Embryos could not be found in six caryopses and 20 of the remaining 25 embryos germinated and grew to mature plants. Five of these plants have since died. Of the twelve plants which have been chromosome counted, ten had the expected chromosome number of $2n = 28$ (Plate 14) and two plants, the only ones with F43 as the female fescue plant, had a chromosome number of $2n = 49$. This chromosome number presumably resulted from an unreduced gamete of the female parent. Only two plants flowered in the following summer (these had $2n = 28$). These two hybrids produced only three inflorescences between them (Plate 14).

Measurements of inflorescence characters of parental species

Plate 14 a) Inflorescence of F. rubra (x 0.6)

b) Inflorescence of F. rubra x V. sicala artificial hybrid
(x 0.8)

c) Inflorescence of V. sicala (x 0.7)

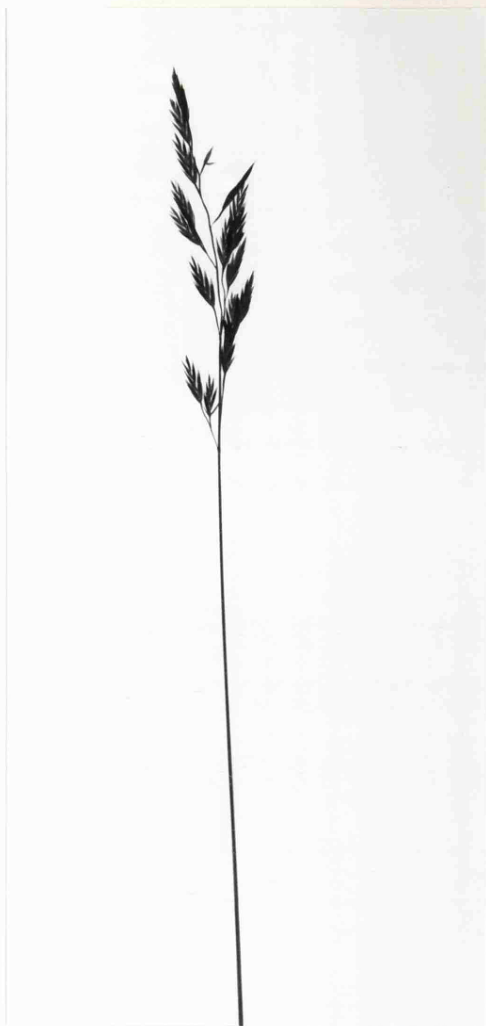
d) Root tip mitosis in F. rubra ($2n = 42$) (x 2,500)

e) Root tip mitosis in F. rubra x V. sicala ($2n = 28$) (x 2,000)

f) Laggards at telophase in F. rubra x V. sicala P.M.C. meiosis
(x 1,800)

g) Tetrad stage of meiosis in F. rubra x V. sicala (x 1,800)

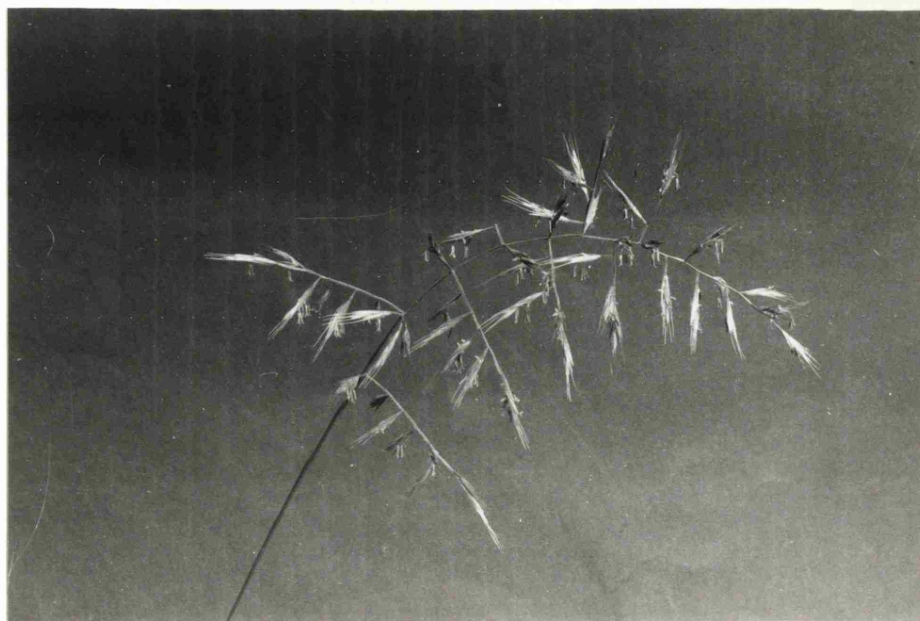
Plate 14



a



b



c

Plate 14 (cont.)

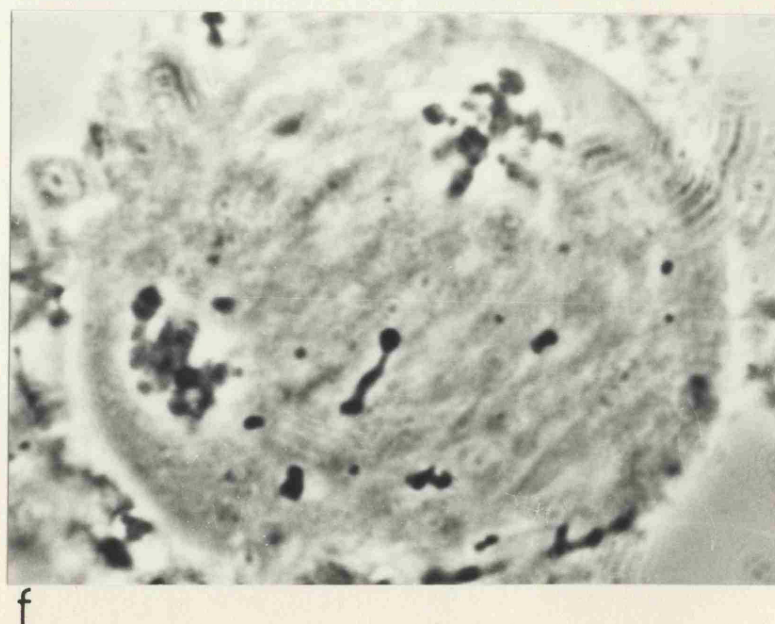
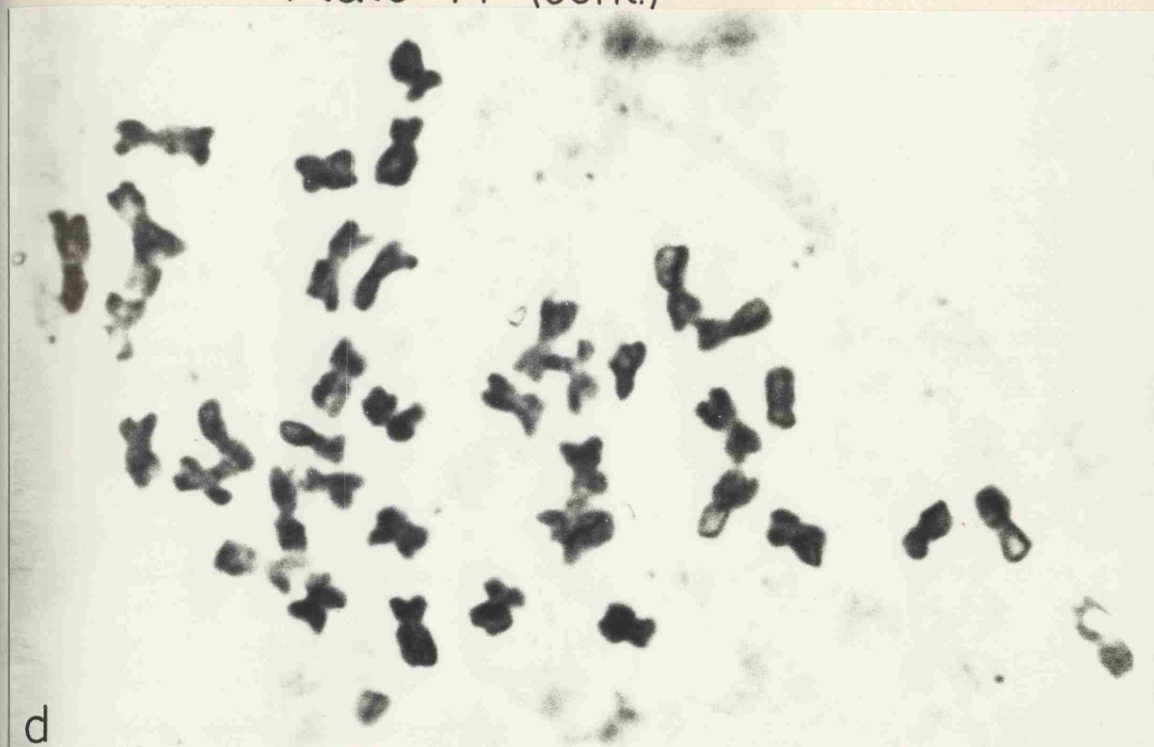


TABLE 27 Measurements of inflorescence characters for F. rubra, V. sicula and F. rubra x V. sicula hybrids

Inflorescence characters	F. rubra	Hybrid	V. sicula
Lower glume length mm	2.2 - 4.1	2.4 - 2.6	0.5 - 2.1
Upper glume length (-awn) mm	3.0 - 5.8	3.9 - 4.5	5.1 - 7.0
Upper glume awn length mm	0 - 0.5	0	0
Lemma length (-awn) mm	4.4 - 7.0	4.4 - 4.6	4.4 - 6.1
Lemma awn length mm	0.1 - 3.3	0.3 - 1.4	2.0 - 3.3
Glume ratio	$\frac{2}{3} - \frac{3}{4}$	$\frac{1}{2} - \frac{2}{3}$	$\frac{1}{10} - \frac{1}{3}$
Anther length mm	2.1 - 3.8	1.8	1.9 - 2.8
Pedicel length mm	1.4 - 3.6	1.2	1.0 - 2.0
Callus length mm	0.2 - 0.3	0.2	0.2
Ovary, pubescent or glabrous	G	G	P
Chromosome number	42	28	14

FIGURE 18. HISTOGRAM OF NUMBER OF BIVALENTS OBSERVED AT MEIOSIS IN F. RUBRA X V. SICULA HYBRIDS.

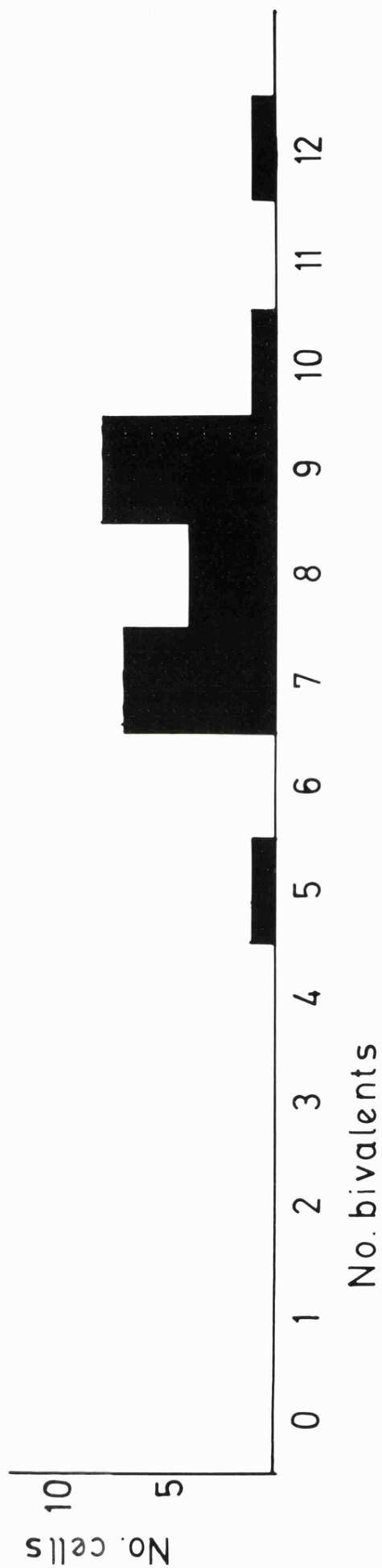
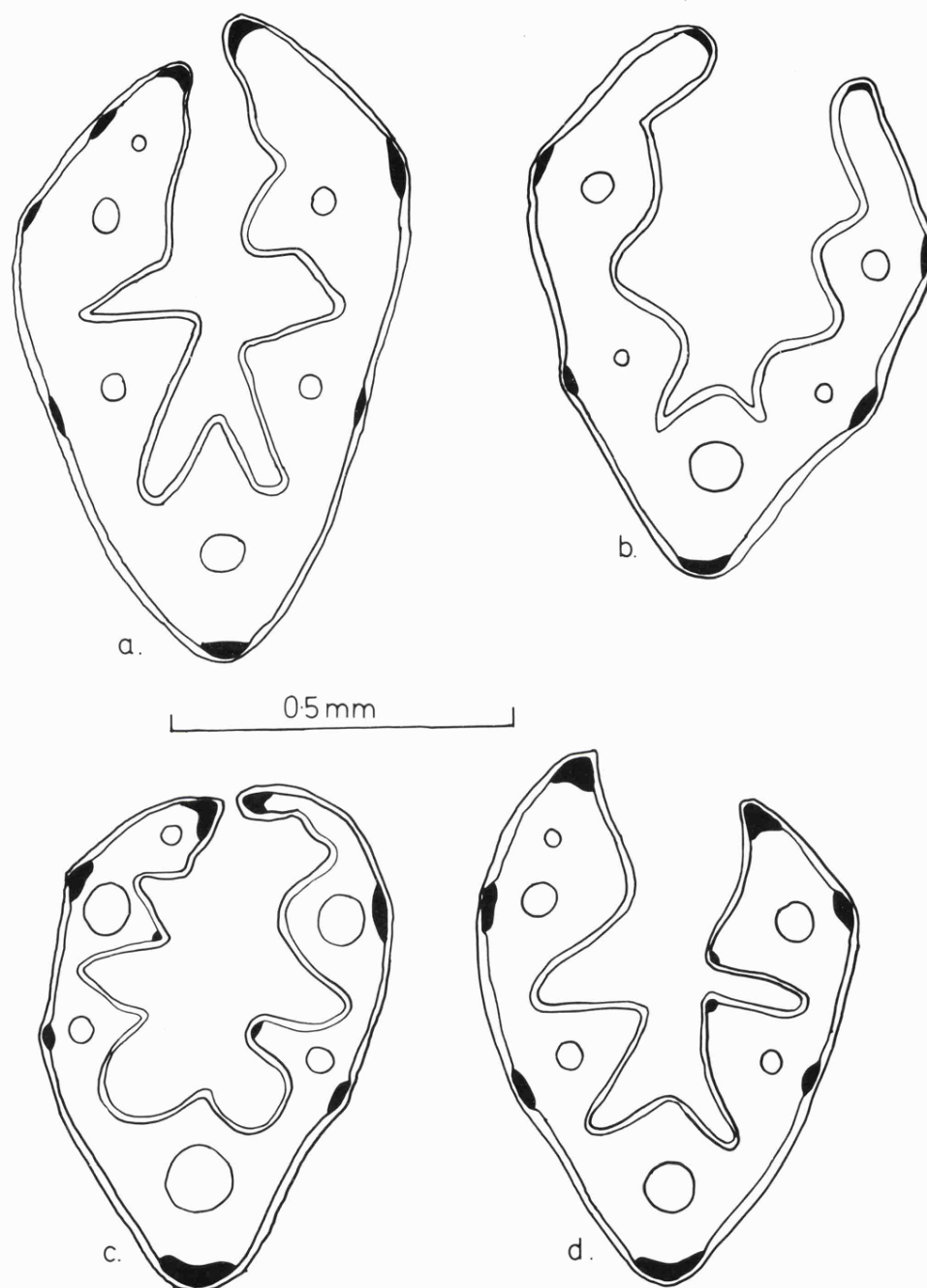


FIGURE 19. INNOVATION LEAF ANATOMY OF V. SICULA, F. RUBRA AND HYBRIDS.



- a. F. rubra $2n=42$
 b. V. sicula $2n=14$
 c. F. rubra X V. sicula $2n=28$
 d. F. rubra X V. sicula $2n=49$

Black = sclerenchyma

Open circle = vascular bundle

and hybrids are given in Table 27. The inflorescence characters were intermediate between those of the parental species except upper glume length which was more similar to F. rubra. The ovary tip was glabrous like that of F. rubra and the anthers in the hybrids were smaller than in either parent. The hybrids had mostly intravaginal branches.

The innovation leaf anatomy of V. sicula, F. rubra (actual parental genotype) and both tetraploid and heptaploid hybrids are given in Fig. 19. V. sicula differs from F. rubra in the size of the ridges corresponding to each vascular bundle; the ridges are much smaller in V. sicula. The bundles of sclerenchyma at the leaf margins are larger in F. rubra than in V. sicula. The tetraploid ($2n = 28$) hybrids are intermediate for size of the ridges but have slightly larger bundles of sclerenchyma than in either parental species (Fig. 19). The two heptaploid ($2n = 49$) hybrids had a ridge size similar to that in F. rubra and a size of sclerenchyma bundles similar to that of the tetraploid hybrids (Fig. 19).

Meiosis was observed in 22 cells and there was a range of five to twelve bivalents, and no trivalents were observed. The mean number of bivalents was 8.2 and the mode being nine bivalents (Fig. 18). The mean chiasmata number per cell was 10.77. The pollen was 100% sterile. The anthers were exerted during flowering but were indehiscent and the hybrid was seed sterile.

15 hybrid plants are still alive. See No. 8 for reciprocal.

2. V. alopecuroides x F. rubra

104 florets were pollinated but no caryopses were produced.

3. V. bromoides x F. rubra

91 florets were pollinated and seven caryopses were produced, all of which were later found to be selfs.

4. V. fontquerana x F. rubra

Four florets were pollinated but no caryopses were produced.

5. V. geniculata x F. rubra

75 florets were pollinated but no caryopses were produced.

6. V. ligustica x F. rubra

74 florets were pollinated and eleven caryopses were produced. Embryos could not be found in nine of the caryopses and the two embryos which were dissected out did not germinate.

7. V. membranacea x F. rubra

224 florets were pollinated and eight caryopses were produced. Embryos could not be found in six of the caryopses and the two embryos which were dissected out did not germinate.

8. V. sicula x F. rubra

201 florets were pollinated and twelve caryopses were produced. Nine embryos were found during dissection of the caryopses; only one of these germinated and it grew to a mature plant. However, the plant has failed to flower in two years and is still alive. The innovation leaf anatomy is similar to that of F. rubra. See No. 1 for reciprocal.

Diploid-octoploid combinations1. V. alopecuroides x F. juncifolia

51 florets were pollinated but no caryopses were produced.

2. V. sicula x F. juncifolia

100 florets were pollinated and 30 caryopses were produced. Three

embryos germinated and one hybrid plant grew to maturity. The chromosome number of this plant was $2n = 35$, as would be expected from a hybrid between these two species. The hybrid did not flower the following summer and the plant died.

3. V. sicula x F. rubra

404 florets were pollinated and 64 caryopses were produced. Of these only two embryos germinated and grew to mature plants. The chromosome number of one plant was counted and found to be the expected number of $2n = 35$. The two plants failed to flower in the following summer and both plants died.

Tetraploid-hexaploid combination

1. V. fasciculata x F. rubra

90 florets were pollinated and 27 caryopses were produced. Embryos could not be found in two caryopses. 23 embryos germinated and all of these grew to mature plants. All 23 hybrids failed to flower the following summer (1978) and were given a cold treatment, soon after which about half flowered. All the hybrids flowered in the following summer (1979) and all had a chromosome number of $2n = 35$.

The hybrids were grown in pots and many produced only a few inflorescences, but a few hybrids were more prolific. The hybrids were all perennial and resembled plants of F. rubra but with long-awned lemmas and one-sided inflorescences (Plate 15). The measurements of inflorescence parts of the parental species and hybrids are given in Table 28. All inflorescence characters were more similar to F. rubra, except anther length and callus length, which were both intermediate. The ovary tip was glabrous as in F. rubra and the pollen grains were smaller than in either parental species.

- Plate 15 a) Inflorescence of V. fasciculata (x 0.8)
- b) Inflorescence of F. rubra x V. fasciculata natural hybrid
(x 0.7)
- c) Inflorescence of F. rubra (x 0.6)
- d) P.M.C. of V. fasciculata x F. rubra with about eleven
bivalents and thirteen univalents at metaphase I (x 2,000)
- e) Giant P.M.C. of V. fasciculata x F. rubra at metaphase I
(x 350)
- f) P.M.C. of V. fasciculata x F. rubra divided into five
segments at meiosis (x 1,800)
- g) Micronuclei in young pollen grains of V. fasciculata x
F. rubra (x 1,800)

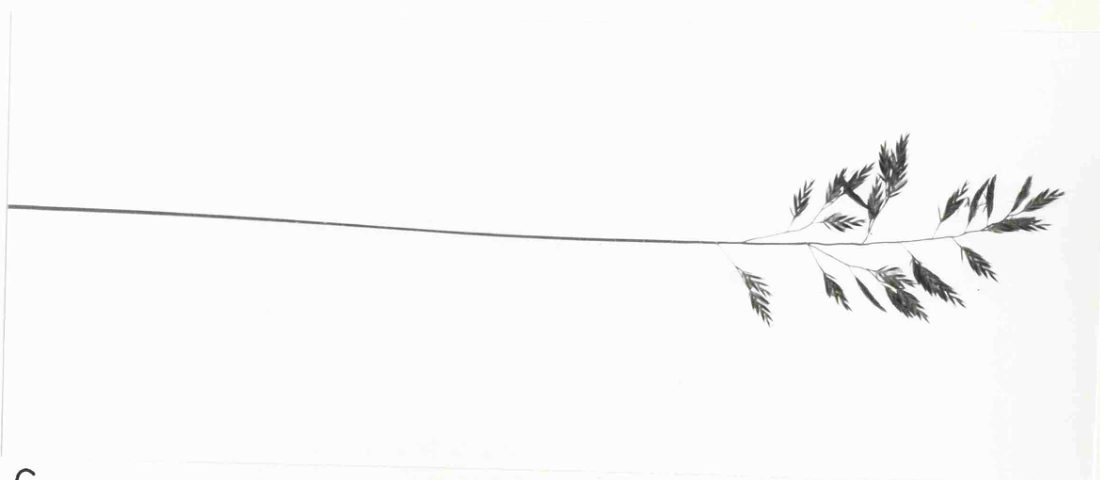
Plate 15



a



b



c

Plate 15 (cont.)



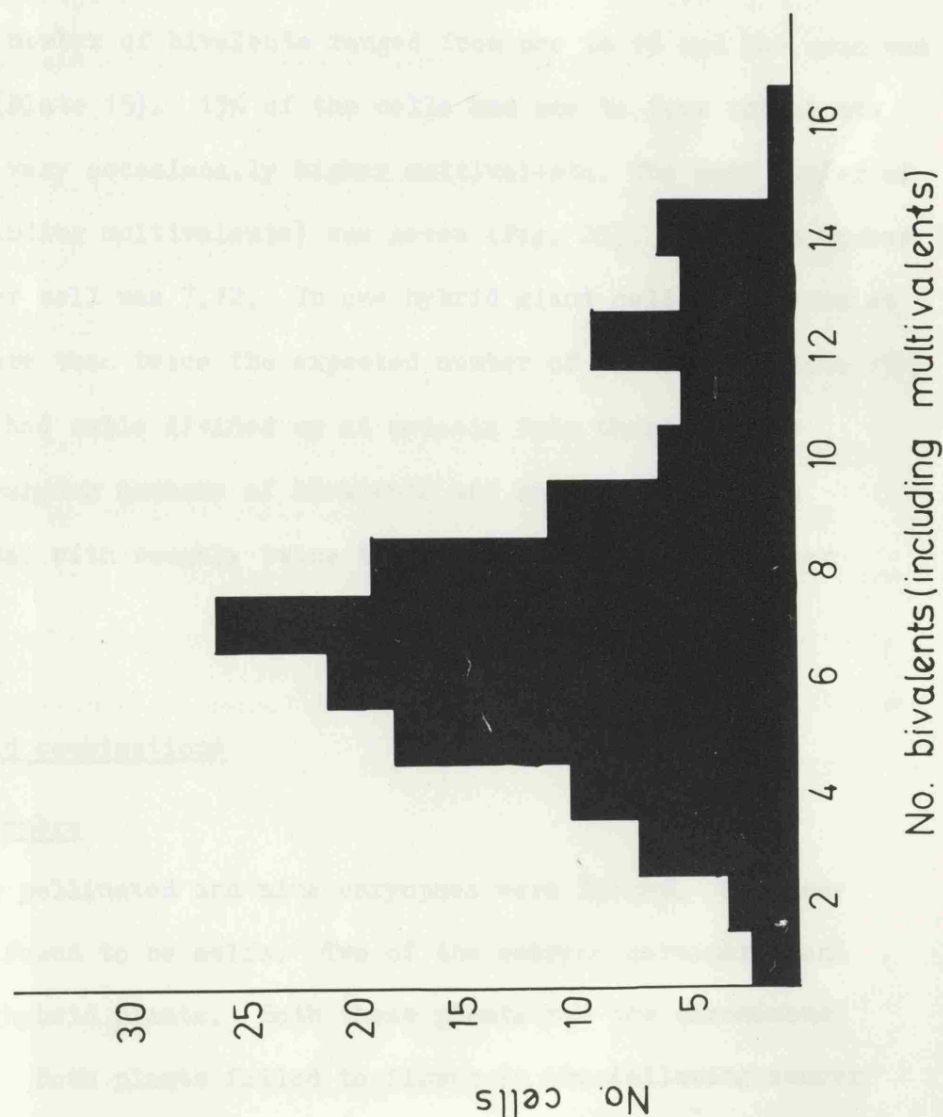
TABLE 28 Measurements of inflorescence characters for V. fasciculata, F. rubra and
V. fasciculata x F. rubra hybrids

Inflorescence characters	V. fasciculata	Hybrid	F. rubra
Lower glume length mm	0.2 - 0.8	2.7 - 3.5	2.2 - 4.1
Upper glume length (-awn) mm	9.5 - 15.9	5.0 - 6.2	3.0 - 5.8
Upper glume awn length mm	1.6 - 11.1	0 - 1.5	0 - 0.5
Lemma length (-awn) mm	8.9 - 16.0	6.0 - 7.0	4.4 - 7.0
Lemma awn length mm	9.8 - 16.4	2.0 - 3.3	0.1 - 3.3
Glume ratio	$\frac{1}{12}$	$\frac{1}{2}$	$\frac{2}{3}$ - $\frac{3}{4}$
Anther length mm	0.6 - 1.1	1.3 - 1.7	2.1 - 3.8
Pedice! length mm	4.0 - 5.6	2.6 - 3.7	1.4 - 3.6
Callus length mm	0.5 - 0.8	0.3 - 0.4	0.2 - 0.3
Ovary, pubescent or glabrous	P	G	G
Pollen grain size μ m	31 - 39	23 - 26	28 - 34
Chromosome number	28	35	42

TABLE 29 Chromosome associations at meiosis in *V. fasciculata* x *F. rubra* hybrids.

No. of quivalents	No. of quadrivalents	No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
			16	3	1
			15	4	1
			14	7	5
			14	6	1
			13	11	1
			13	9	2
		2	11	7	1
		1	12	8	1
			12	11	4
			12	10	3
		3	9	7	1
		2	10	9	1
			11	15	1
			11	14	2
			11	13	1
		1	10	12	1
			10	16	2
			10	15	3
			10	14	1
			9	19	3
			9	18	1
			9	17	3
			9	15	1
		1	8	18	1
		1	8	16	2
			8	22	1
			8	20	2
			8	19	6
			8	18	1
			8	17	1
			8	16	1
			8	14	1
		4	4	23	1
	1		7	18	2
	1		7	17	3
			7	24	1
			7	21	15
			7	19	2
			7	18	1
			7	14	4
1			6	18	1
		1	6	21	1
		1	6	20	1
			6	25	2
			6	23	12
			6	22	1
			6	21	2
			6	20	1
			6	18	1
		2	4	21	1
		1	5	22	1
			5	26	1
			5	25	9
			5	23	2
			5	20	2
	1	1	3	22	1
		1	4	26	1
		1	4	25	1
		1	4	22	1
			4	30	1
			4	27	7
			4	24	1
		1	3	26	1
			3	29	4
			3	27	1
		1	2	28	2
			2	31	1
			2	30	1
			2	28	1
			1	33	1
			1	31	1

FIGURE 20. HISTOGRAM OF NUMBER OF BIVALENTS (INCLUDING MULTIVALENTS) OBSERVED AT MEIOSIS IN V. FASCICULATA X F. RUBRA HYBRIDS.



60 cells were observed at meiosis. The pollen was 97.5 - 100% sterile. The florets opened during flowering and the anthers were exerted but they were indehiscent and all the hybrids were seed sterile.

Artificial hybrids made by R. Cotton in 1973 (Stace & Cotton, 1974) were also examined cytologically. 90 cells were observed at meiosis. The combined cytological data for both the hybrids made during this period of research and those made by R. Cotton are presented in Table 29. The number of bivalents ranged from one to 16 and the mean was 7.2 bivalents (Plate 15). 13% of the cells had one to four trivalents and there were very occasionally higher multivalents. The mode number of bivalents (including multivalents) was seven (Fig. 20). The mean number of chiasmata per cell was 7.12. In one hybrid giant cells were seen at meiosis with more than twice the expected number of bivalents (Plate 15). Another hybrid had cells divided up at meiosis into three or five segments with varying numbers of bivalents and univalents in each segment, in total with roughly twice the expected chromosome number (Plate 15).

Hexaploid-hexaploid combinations

1. V. myuros x F. rubra

67 florets were pollinated and nine caryopses were formed. Of these two were later found to be selfs. Two of the embryos germinated and grew to mature hybrid plants. Both these plants had the chromosome number $2n = 42$. Both plants failed to flower in the following summer and were given a cold treatment and still no inflorescences were produced. One plant died and the other plant is still alive but has not flowered over two years.

c. Interspecific *Festuca* crosses

Hexaploid-octoploid combinations

1. *F. juncifolia* x *F. rubra* subsp. *rubra* ($2n = 42$)

154 florets were pollinated and 36 caryopses were produced. Embryos were dissected out of 33 caryopses and 23 of them germinated. 16 grew to mature plants. The death of the seven seedlings was probably due to insufficient root growth in the agar medium. Eleven plants were chromosome counted and had the expected $2n = 49$ (Plate 16). All the hybrids failed to flower in the following summer (1979).

The innovation leaf anatomy of parents and hybrids is given in Fig. 21. *F. juncifolia* has more vascular bundles and ridges than *F. rubra*. *F. juncifolia* has more or less continuous sclerenchyma on the abaxial side often forming girders of sclerenchyma with the vascular bundles, it also has bundles of sclerenchyma in each ridge. *F. rubra* has discrete bundles of sclerenchyma corresponding to each vascular bundle and rarely has any sclerenchyma in the ridges. The hybrids have the same number of vascular bundles as *F. rubra* and the sclerenchyma is broken and intermediate in amount between the parental species, but the sclerenchyma does not form girders. The hybrids have no sclerenchyma in the ridges.

16 hybrid plants are still alive.

2. *F. rubra* subsp. *arenaria* ($2n = 56$) x *F. rubra* subsp. *rubra* ($2n = 42$)

30 florets were pollinated and six caryopses were produced. Three caryopses germinated and two grew to mature plants. The chromosome number of both plants was $2n = 49$. Both plants failed to flower in the following summer (1979).

The innovation leaf anatomy of parents and hybrids is given in Fig. 21. *F. rubra* subsp. *arenaria* and *F. rubra* subsp. *rubra* have the

Plate 16 Root tip mitosis in F. juncifolia x F. rubra ($2n = 49$)
(x 2,000)

Plate 16

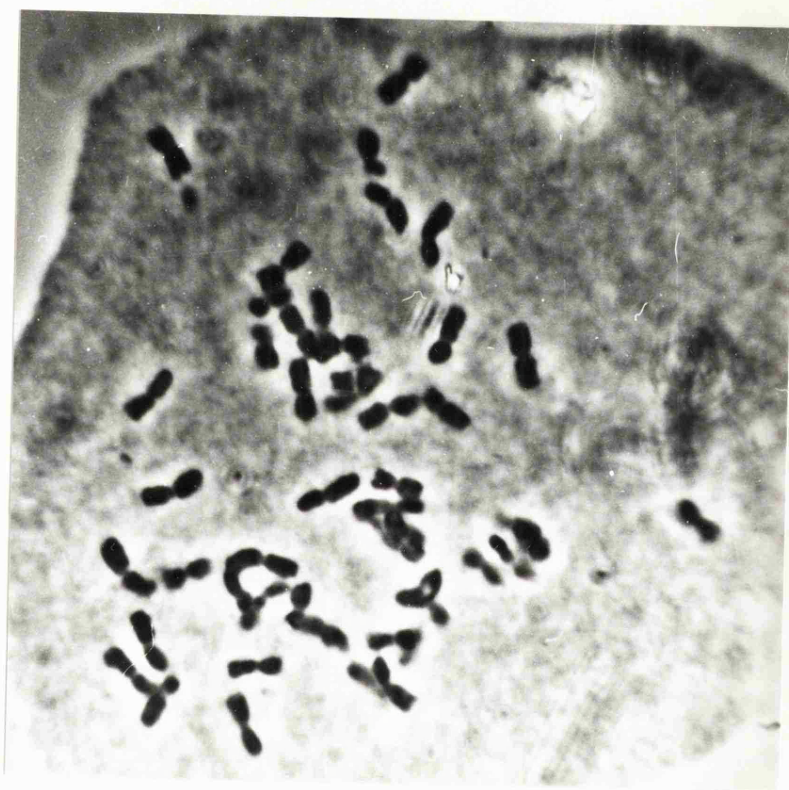
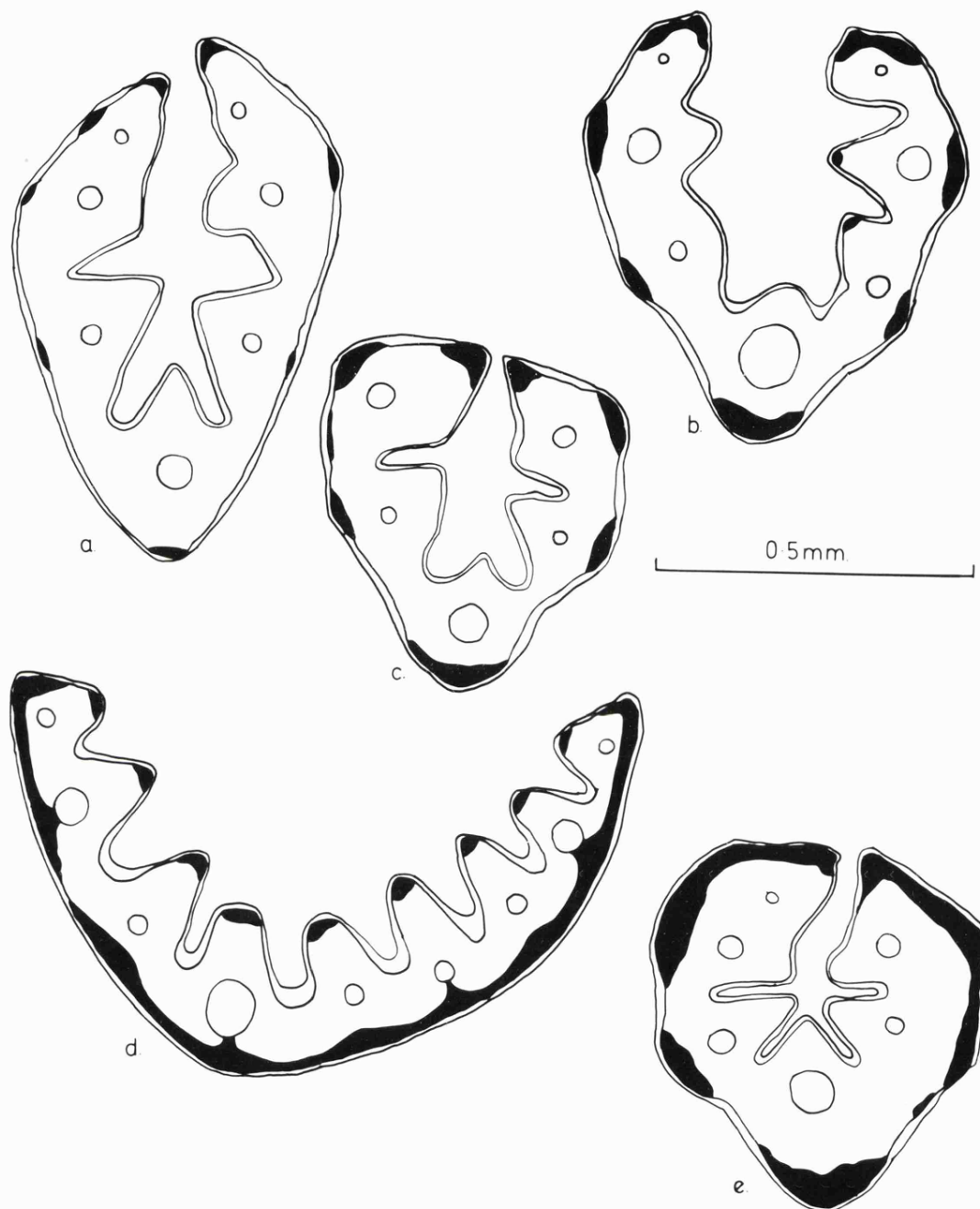


FIGURE 21. INNOVATION LEAF ANATOMY OF F. JUNCIFOLIA, F. RUBRA AND HYBRIDS.



- a. F. rubra
- b. F. rubra subsp. arenaria
- c. F. rubra subsp. arenaria X F. rubra subsp. rubra
- d. F. juncifolia
- e. F. juncifolia X F. rubra subsp. rubra

same distribution of adaxial sclerenchyma but subsp. arenaria has larger bundles and occasionally has small amounts of sclerenchyma in the ridges. The hybrids are intermediate between the parents for the amount of adaxial sclerenchyma and have no sclerenchyma in the ridges.

Both hybrid plants are still alive.

v. Summary of cytological data

A summary of the cytological data for parental species and artificial hybrids is given in Table 31. The six diploid species of Vulpia all had seven bivalents at meiosis. The five diploids for which data were obtained all had a mean of over 1.79 chiasmata per bivalent. V. fasciculata had 13 to 14 bivalents and a mean of 1.9 chiasmata per bivalent. F. rubra and F. juncifolia had 27 and 28 bivalents respectively. Both diploid and polyploid species were exclusively bivalent formers.

Of the diploid hybrid ⁶V. ligustica x V. geniculata had the highest number of bivalents per cell (mean = 6.20) and the highest chiasmata number per bivalent (mean = 1.85), both similar to the values of the parental species. The diploid hybrids with the lowest mean number of bivalents were V. fontquerana x V. ligustica and V. membranacea x V. sicula with 4.43 and 4.47 respectively; these hybrids also had a low number of chiasmata per bivalent. All the diploid hybrids were able to form up to seven bivalents and in two diploid hybrids a very low number of trivalents was observed.

Of the triploid hybrids, V. fasciculata x V. ligustica had the highest number of bivalents (mean = 6.28) and V. membranacea x V. fasciculata had the highest number of trivalents (mean = 0.69). The lowest number of bivalents (mean = 5.31) was found in V. fasciculata x V. alopecuroides. In all the triploid hybrids some cells had over seven bivalents, and in all the triploids trivalents were observed though the

TABLE 30 Performance of F_1 artificial hybrids

Artificial hybrids	Vigour of F_1 plant		No. of months to flowering	Pollen sterility	Plant fertility	Chromosome number
	Vegetative growth	No. of inflorescences				
V. fontquerana x V. ligustica	Poor	Few	6	100%	Sterile	14
V. ligustica x V. geniculata	Vigorous	Many	2	98 - 100%	Sterile	14
V. membranacea x V. geniculata	Vigorous	Many	2	100%	Sterile	14
V. membranacea x V. ligustica	Medium	Many produced over several months	6	100%	Sterile	14
V. membranacea x V. sicula	Poor	Few	7	100%	Sterile	14
V. sicula x V. geniculata	Vigorous	Many	3	100%	Sterile	14
V. fasciculata x V. alopecuroides	Vigorous	Many	3	100%	Sterile	21
V. fasciculata x V. geniculata	Vigorous	Many	2	100%	Sterile	21
V. fasciculata x V. ligustica	Vigorous	Many	2	100%	Sterile	21
V. fasciculata x V. membranacea	Vigorous	Many	3	100%	Sterile	21
V. fasciculata x V. sicula	Poor	Very few	5	100%	Sterile	21
V. membranacea x V. fasciculata	Vigorous	Many	3	100%	Sterile	21
F. rubra (2n=42) x V. sicula	Medium	Very few	10 to ?	100%	Sterile	28
F. rubra (2n=42) x V. sicula	Medium	Not flowered after one year				49
V. sicula x F. rubra (2n=42)	Medium	Not flowered after one year				28
V. sicula x F. rubra (2n=56)	Medium	Not flowered after one year				35
V. sicula x F. juncifolia	Medium	Not flowered after one year				35
V. fasciculata x F. rubra (2n=42)	Medium	Few to many	12 to 24	97.5 - 100%	Sterile	35
V. myuros x F. rubra (2n=42)	Poor	Not flowered after two years				42
F. juncifolia x F. rubra (2n=42)	Medium	Not flowered after one year				49
F. rubra subsp. arenaria x F. rubra subsp. rubra (2n=42)	Medium	Not flowered after one year				49

mean number of trivalents was very low. Some triploid hybrids had very low numbers of higher multivalents.

F. rubra x V. sicula ($2n = 28$) had the highest number of bivalents of all the hybrids (mean = 8.18) but a low chiasmata number per bivalent (mean = 1.28). V. fasciculata x F. rubra ($2n = 35$) had a lower number of bivalents (mean = 7.24) and chiasmata per bivalent (mean = 1.17).

vi. Summary of morphological data

A summary of the inheritance of morphological characters in the F_1 artificial hybrids is given in Table 32. Where the parental species differ in the measurement of a character, in just over half the cases the hybrids had an intermediate measurement; in the other cases either the character was more similar to one parent or occasionally it was different from both parents.

Lower glume length was either intermediate or closer to that of the larger parent; anther length was either intermediate or closer to that of the shorter parent; pedicel length was closer to that of the shorter parent; pubescent ovaries were dominant to glabrous ovaries in interspecific hybrids but the reverse was true in intergeneric hybrids; and pollen grains were nearly always smaller than in either parent. The rest of the characters differed in their expression in different F_1 hybrids, and no general patterns relating to particular species used as parents or to the direction of the cross can be discerned, except in the case of intergeneric hybrids involving F. rubra. Here, either the F. rubra attributes were dominant or the hybrid attributes were intermediate between those of the parents.

All the hybrid attributes were within the range of the parental species except for the following: pollen grain size was smaller

TABLE 32 Summary of the inheritance of morphological characters in the F_1 artificial hybrids.

	2n	Lower glume length	Upper glume length (awn)	Upper glume length (awn)	Lemna length (awn)	Lemna awn length	Glume ratio	Anther length	Pedicle length	Callus length	Ovary pubescent or glabrous	Pollen grain size	Annual or perennial
<i>V. fontquerana</i> x <i>V. ligustica</i>	14	I	I	I	L (shorter)	L (shorter)	I	I	I	L (shorter)	L (P)		
<i>V. ligustica</i> x <i>V. geniculata</i>	14	I	I	I	L (longer)	L (longer)	I					smaller than parents	
<i>V. membranacea</i> x <i>V. geniculata</i>	14	G (longer)	M (longer)	I	I	M (longer)	G	M (shorter)		I	G (P)	smaller than parents	
<i>V. membranacea</i> x <i>V. ligustica</i>	14	I	I	I	L (shorter)	L (shorter)		M (shorter)		I	L (P)		
<i>V. membranacea</i> x <i>V. sicula</i>	14	I	I	I	S (shorter)	S (shorter)		I	S (shorter)	I	S (P)	smaller than parents	M & S (either)
<i>V. sicula</i> x <i>V. geniculata</i>	14	G (longer)					G	shorter than parents	S (shorter)				?
<i>V. fasciculata</i> x <i>V. aiopaeuros</i>	21	F (longer)	F (longer)	F (longer)	F (longer)	F (longer)		I			F (P)		
<i>V. fasciculata</i> x <i>V. geniculata</i>	21	I	F (longer)	G (shorter)	I (longer)	F (longer)	I	I		I		smaller than parents	
<i>V. fasciculata</i> x <i>V. ligustica</i>	21	F (longer)	F (longer)	F (longer)	F (longer)	F (longer)		I		I		smaller than parents	
<i>V. fasciculata</i> x <i>V. sicula</i>	21	I	I	I	I	I		I	S (shorter)	I		smaller than parents	F (annual)
<i>V. membranacea</i> x <i>V. fasciculata</i>	21	I	I	I	I	I				longer than parents		smaller than parents	
<i>F. rubra</i> x <i>V. sicula</i>	28	I (shorter)	R (shorter)				I	shorter than parents			R (G)		
<i>V. fasciculata</i> x <i>F. rubra</i>	35	R (longer)	R (shorter)	R (shorter)	R (shorter)	R (shorter)	I	I		I	R (G)	smaller than parents	R (perennial)

KEY

I - where the attribute of the hybrid is intermediate between that of the parental species.

P, G, L, M, R, S - correspond to the name of the parental species to which the hybrid is more similar in the character concerned.

The information in brackets indicates the relative characters of the parent which the hybrid resembles.

(G) and (P) - glabrous and pubescent respectively.

than in both parental species for every F_1 hybrid measured except V. sicula x V. geniculata; anther length was smaller than in both parental species in V. sicula x V. geniculata and F. rubra x V. sicula; and callus length was slightly longer in V. membranacea x V. fasciculata than in either parental species.

5.3 Natural intergeneric hybrids

1. F. rubra x V. bromoides

This hybrid has been collected in 1969, 1973 and 1976 from the same locality (Shingle Street, Hollesley in E. Suffolk) by P.J.O. Trist. In 1969, Trist (pers. comm.) found ten hybrid plants growing over an area of 40 to 50 sq. yds, in 1973; he found only two hybrid plants; in 1974 and 1975 he found none, and in 1976 he found only one, growing in the same position as one of the 1973 hybrids. Part of the hybrid plant found in 1976 is now growing at Leicester.

The hybrids are perennial. The specimen growing at Leicester produced a few inflorescences in the summer of 1978 but failed to flower in the following year.

The measurements of inflorescence characters of the parental species and of two hybrid specimens, one collected in 1976 and growing at Leicester and the other a pressed specimen collected by Trist in 1973, are given in Table 33. The hybrids are intermediate between the parental species for lemma awn length, and anther length and number, whereas in the remaining inflorescence characters the parental species are very similar (Plate 17).

The chromosome number of the specimen grown at Leicester was counted and was between 40 and 45. This agrees with the number of chromosomes deduced from the number of multivalents, bivalents and

TABLE 33 Measurements of inflorescence characters for F. rubra, V. bromoides and F. rubra x V. bromoides natural hybrids

Inflorescence characters	F. rubra	F. rubra x V. bromoides hybrid growing at Leicester	F. rubra x V. bromoides collected from Shingle Street	V. bromoides
Lower glume length mm	2.2 - 4.1	2.5 - 3.0	2.4 - 3.0	2.5 - 5.0
Upper glume length (-awn) mm	3.0 - 5.8	4.5 - 5.5	4.0 - 5.0	4.5 - 6.5
Upper glume awn length mm	0 - 0.5	0	0	0 - 2.0
Lemna length (-awn) mm	4.4 - 7.0	6.0 - 7.0	5.0 - 7.0	4.5 - 7.5
Lemna awn length mm	0.1 - 3.3	3.5 - 5.0	2.2 - 4.3	6.0 - 10.0
Glume ratio	$\frac{2}{8} - \frac{3}{4}$	$\frac{1}{2} - \frac{3}{4}$	$\frac{1}{2} - \frac{3}{4}$	$\frac{1}{2} - \frac{3}{4}$
Anther length mm	2.1 - 3.8	1.0*	1.2 - 1.5	0.4 - 0.7
Pedicel length mm	1.4 - 3.6	1.2 - 3.0	1.5 - 2.0	0.5 - 3.5
Callus length mm	0.2 - 0.3	0.2	0.2	0.2
Ovary pubescent or glabrous	G	G	G	G

* Measurement of immature anthers

Plate 17 a) Pressed specimen of natural F. rubra x V. bromoides hybrid
(x 0.5)

b) Inflorescences of V. bromoides (x 0.5):

See Plate 15c for inflorescence of F. rubra

c) & d) P.M.C. of F. rubra x V. bromoides at metaphase I
(x 1,800)

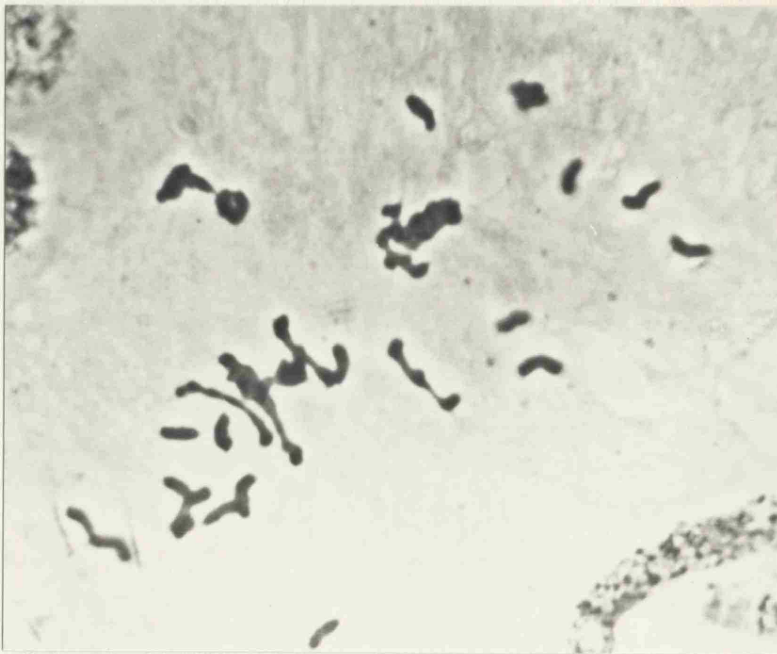
Plate 17



Plate 17 (cont.)



b



c



d

TABLE 34 Chromosome associations at meiosis in *F. rubra* x *V. bromoides* ($2n = c42$) natural hybrid

No. of quivalent	No. of quadrivalents	No. of trivalents	No. of bivalents	No. of univalents	No. of cells observed
1		1	14	14	1
		3	13	c 9	1
			10	20	1
			7	12	1
			10	c17	1
		2	6	16	1
		2	7	23	1
			7	25	1
		2	5	22	1
		1	6	27	2

univalents observed at meiosis, which was either 42 or 43. This chromosome number of about $2n = 42$ is obviously not what is expected from the hybridisation of diploid V. bromoides ($2n = 14$) with hexaploid F. rubra ($2n = 42$).

Observations were made on eleven cells at meiosis and the results are given in Table 34 (Plate 17). The following are the ranges of the chromosome associations; 0 to 1 quinquivalents, 0 to 1 quadri-valents, 0 to 3 trivalents, 5 to 14 bivalents and 9 to 27 univalents. There were mostly rod bivalents and the pollen is 99% sterile and the hybrid was seed sterile.

2. F. rubra x V. myuros

The hybrid plant collected from Snettisham, Norfolk, by R.P. Libbey in 1974 is growing at Leicester. This perennial hybrid produced only a few inflorescences in 1978 and failed to flower in 1979 and no pressed specimen was made. The measurements of inflorescence characters of the hybrid in Table 35 are taken from a pressed specimen of a hybrid collected from Stockport, Cheshire by C.A. Stace and R. Cotton in 1974. The general appearance of the hybrid is very similar to that of V. myuros with a long slender inflorescence and with the whole inflorescence reddish when mature (Plate 18). The lower glume is more similar to that of V. myuros. The anther length is intermediate and for the remaining inflorescence characters the parental species are very similar.

The chromosome number of the hybrid plant growing at Leicester is $2n = 42$. The first metaphase of meiosis was not found, but the pollen was 100% sterile.

TABLE 35 Measurements of inflorescence characters for F. rubra, V. myuros and F. rubra xV. myuros hybrid

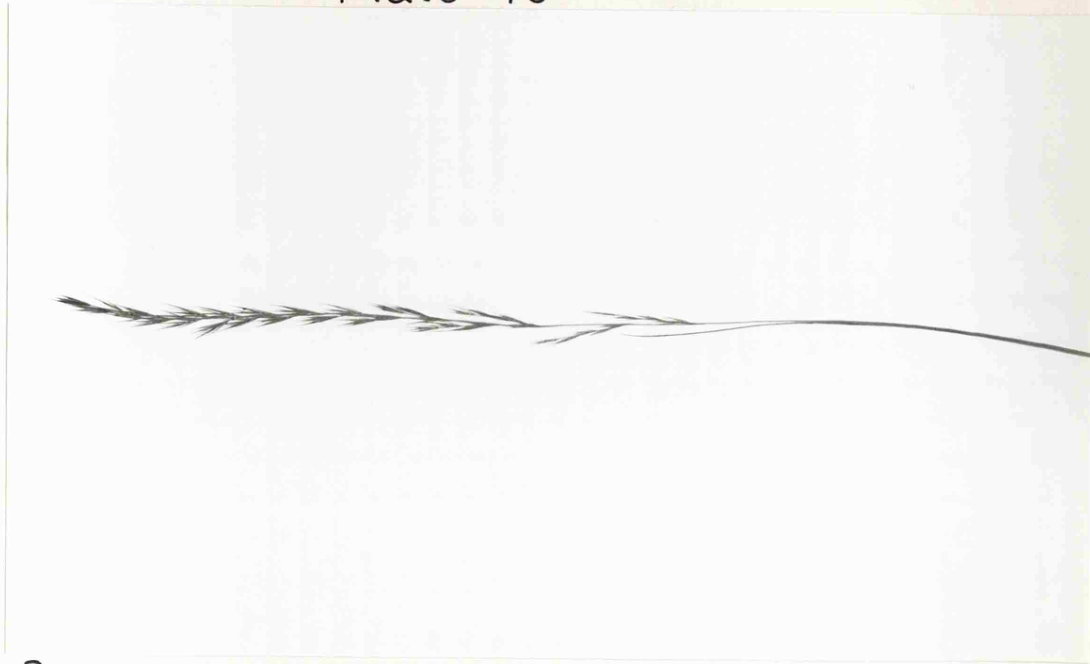
Inflorescence characters	<u>F. rubra</u>	<u>F. rubra</u> x <u>V. myuros</u> hybrid collected from Stockport	<u>V. myuros</u>
Lower glume length mm	2.2 - 4.1	2.5 - 3.6	0.5 - 2.5
Upper glume length (-awn) mm	3.0 - 5.8	4.5 - 6.5	2.5 - 5.0
Upper glume awn length mm	0 - 0.5	0	0 - 1.0
Lemma length (-awn) mm	4.4 - 7.0	6.0 - 7.0	4.5 - 7.5
Lemma awn length mm	0.1 - 3.3	5.3 - 7.0	5.5 - 10.0
Glume ratio	$\frac{2}{3} - \frac{4}{5}$	$\frac{1}{2} - \frac{3}{4}$	$\frac{1}{10} - \frac{2}{5}$
Anther length mm	2.1 - 3.8	1.0 - 1.5	0.4 - 0.8
Pedicel length mm	1.4 - 3.6	1.0 - 2.5	0.4 - 2.0
Callus length mm	0.2 - 0.3	0.2	0.2
Ovary, pubescent or glabrous	G	G	G

Plate 18 a) Inflorescence of V. myuros (x 0.7)

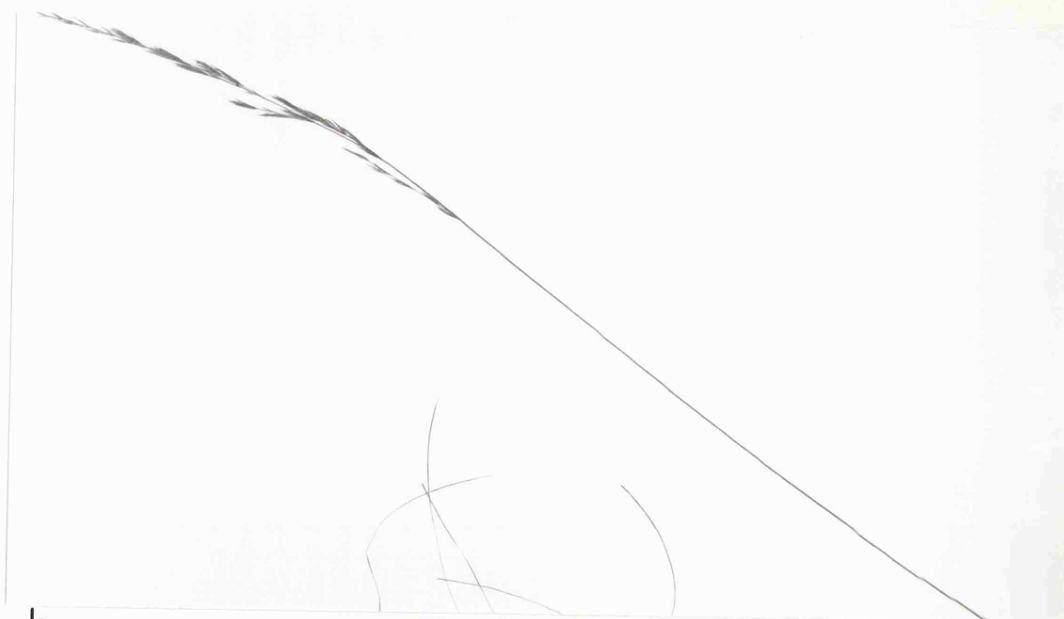
b) Inflorescence of natural F. rubra x V. myuros hybrid
(x 0.5)

See Plate 15c for inflorescence of F. rubra

Plate 18



a



b

3. F. rubra x V. fasciculata

F. rubra x V. fasciculata hybrids collected from eight localities in England and Wales by C.A. Stace and R. Cotton are growing at Leicester. The chromosome numbers were counted and were found to be $2n = 35$. Cotton and Stace (1974) have previously examined the morphology of these hybrids and presented a table of measurements of inflorescence characters (Table 36). The hybrids are intermediate between their parental species in lemma length. The lower glume length, upper glume awn and lemma awn are closer to that of the F. rubra parent. The leaf anatomy of the hybrids is intermediate between that of the parental species. Sclerenchyma is usually present below all the vascular bundles but is never as well developed as in F. rubra. The midrib in the hybrids is keeled to a small degree.

Stace and Cotton (1974) reported that the stainable pollen is often 0% and they never observed it above 3%. The anthers were found to be indehiscent. Stace and Cotton (1974) found at meiosis ten to 14 bivalents, with only two or three of these being ring bivalents. In the present study 18 cells were observed at meiosis (Table 37) and the number of bivalents ranged from two to 14; most of these were rod bivalents.

Inflorescences of the natural hybrids were collected and about 1,500 florets from each were sown. A single F_2 plant was obtained from the hybrid from Berrow, Somerset. The plant has not produced any inflorescences over two years. Cells with chromosome numbers of 41 and 45 were observed in this plant (Plate 19). The F_2 plant is still alive but not very vigorous.

TABLE 36 Floral measurements of *Festuca rubra*, *F. juncifolia*, *Vulpia membranacea* and hybrids. (Taken from Stace & Cotton, 1974).

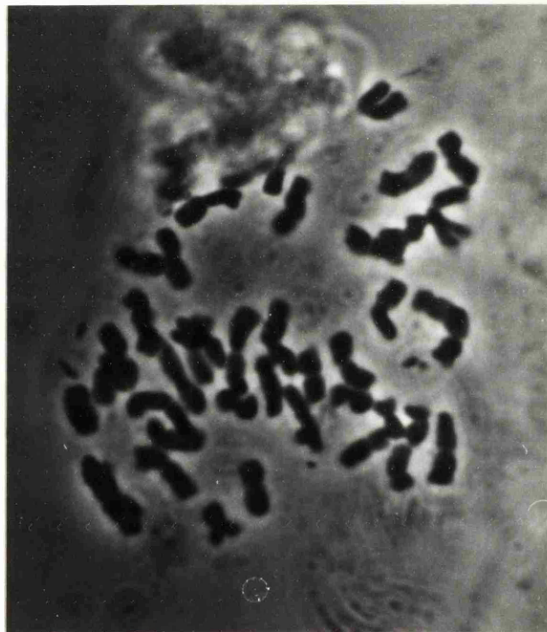
	Mean length in mm (10 measurements per plant)			
	Lower Glume	Upper Glume	Lemma	Lemma- awn
<i>F. rubra</i>	2.2 - 4.1	3.0-5.8(-7.3)	4.4-7.0(-7.7)	0.1 - 3.3
<i>F. juncifolia</i>	4.2 - 6.7	5.5 - 8.6	6.5 - 8.9	0.1 - 2.1
Hybrid (pentaploid)	2.4 - 4.4	5.2 - 8.0	6.0 - 9.5	2.0 - 5.5
Hybrid (hexaploid)	3.5 - 7.2	8.0 -11.5	9.5 -10.5	3.5 - 5.0
<i>V. membranacea</i>	0.2 - 2.6	12.8 -32.0 (10.7-19.3 excluding awn)	10.7 -18.3	8.0 -25.2

TABLE 37 Chromosome associations of meiosis in *F. rubra* x *F. fasciculata* hybrids

No. of bivalents	No. of univalents	No. of cells observed
14	7	1
12	11	1
9	17	1
8	19	3
7	21	3
6	22	2
5	25	4
4	27	1
3	29	1
2	31	1

Plate 19 Root tip mitosis in F_2 of F. rubra x V. fasciculata ($2n = c45$)
(x 2,500)

Plate 19



4. F. juncifolia x V. fasciculata

Stace and Cotton (1974) found a hexaploid hybrid at Littlehampton, W. Sussex, and have presented floral measurements of this hybrid (Table 36). The hybrid is now growing at Leicester. The hexaploid hybrid differs from the pentaploid hybrids in its longer upper glumes, lemmas and (to a lesser extent) lower glumes (Plate 20). The hexaploid hybrid has only extravaginal branches, as in F. juncifolia, whereas V. fasciculata has only intravaginal branches.

In the present study inflorescences were picked for meiosis but only two cells were observed; both cells had 18 bivalents and six univalents.

5.4 Chromosome doubling of diploid species of Vulpia

The results of this experiment are given in Table 38. Of the 56 seedlings which survived the colchicine treatment 24 (57%) had a doubled up chromosome number in the root tips, but only four plants of V. geniculata and one of V. alopecuroides showed a doubled chromosome number in the inflorescence.

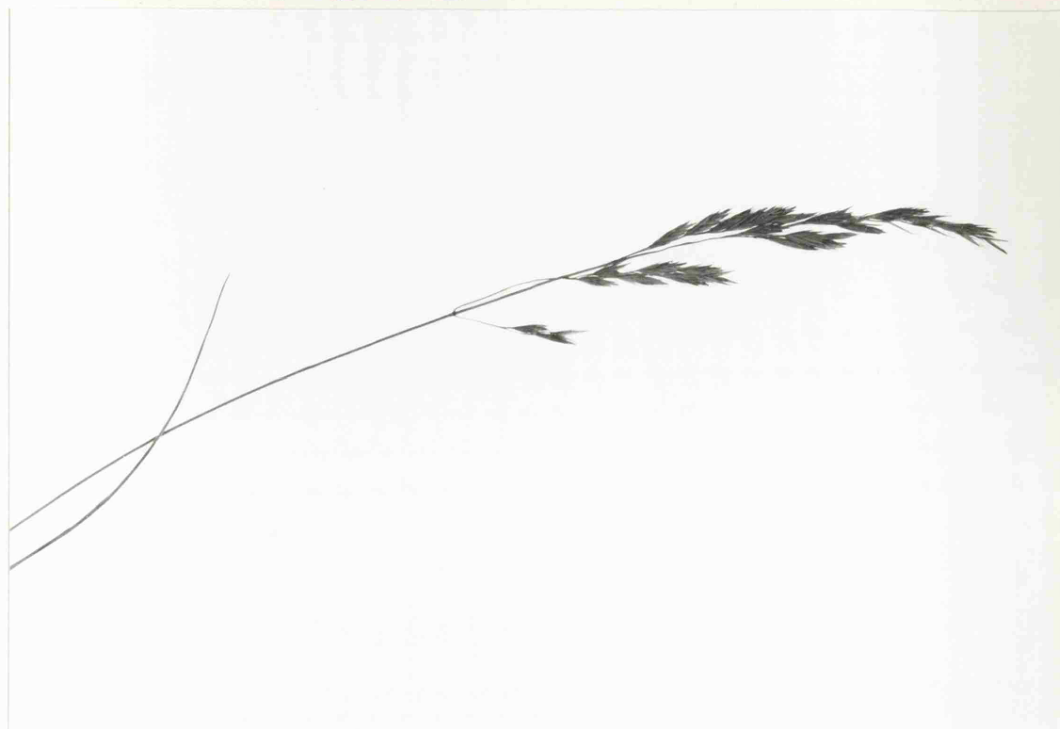
44 cells were observed at meiosis in a doubled up inflorescence of V. geniculata (Table 39). The number of quadrivalents ranged from 0 to 7 and the number of bivalents ranged from 0 to 14 (Plate 21). No autotetraploid caryopses were collected from these plants, presumably because the mixture of both quadrivalents and bivalents in most cells at meiosis resulted in sterile pollen. Thus this experiment was not successful in producing autotetraploids from diploid annual species of Vulpia.

Plate 20 a) Inflorescence of F. juncifolia (x 0.6)

b) Inflorescence of F. juncifolia x V. fasciculata natural
hybrid (x 0.7)

See Plate 15a for inflorescence of V. fasciculata

Plate 20



a



b

TABLE 38 Chromosome doubling experiment on four diploid species of *Vulpia*.

Species and genotypes	No. of seedlings treated	No. of seedlings surviving treatment	No. of plants (no. of plants that died later)	No. of plants with doubled chromosome no. in inflorescence
<i>V. alopecuroides</i> V240	22	15	3 (1)	1
<i>V. geniculata</i> V82	25	8	4	4
<i>V. ligustica</i> V255	15	3	1	0
<i>V. membranacea</i> V5	20	11	8 (3)	0
V36	24	13	4 (1)	0
V364	18	6	4 (3)	0

TABLE 39 Chromosome associations at meiosis in autotetraploid
V. geniculata ($2n = 28$).

No. of quadrivalents	No. of bivalents	No. of cells observed
0	14	5
1	12	6
2	10	10
3	8	6
4	6	11
5	4	3
6	2	2
7	0	1

Plate 21 a) Root tip mitosis in artificially produced autotetraploid of

V. membranacea ($2n = 28$) (x 2,000)

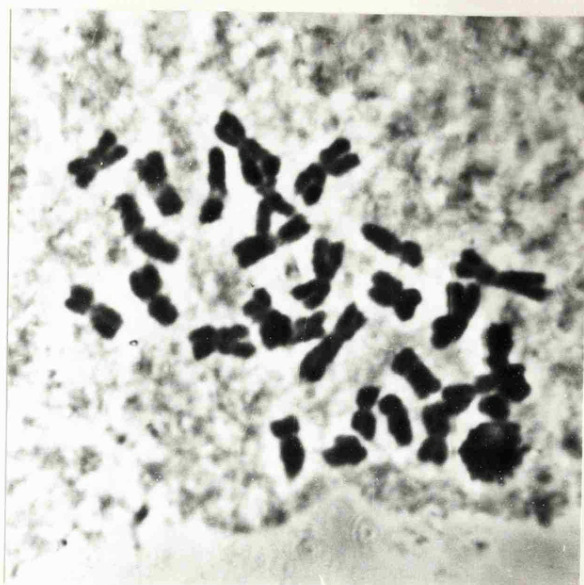
b) P.M.C. of artificially produced autotetraploid of

V. geniculata with 14 bivalents at metaphase I (x 2,500)

c) P.M.C. of artificially produced autotetraploid of

V. geniculata with five quadrivalents and four bivalents at metaphase I (x 2,500)

Plate 21



a



b



c

5.5 Giemsa staining

The scheme was followed as outlined in Chapter 4. Several runs following this method produced some root tip squashes where faint bands could be seen. The schedule was altered by varying the time in HCl and the strength of the HCl, by omitting the immersion of slides in 45% acetic acid, by pretreating with gammexane instead of chilled water, by varying the time in 3:1, by varying the strength of the barium hydroxide solution, and by varying the length of time of the air-drying stages, as found in other schedules for Giemsa staining. None of these alterations to the schedule made the staining of the bands more intense.

Very faint bands were seen in the chromosomes of V. geniculata, V. ligustica and V. membranacea. Terminal bands occurred in some chromosomes of all three species and some chromosomes of V. ligustica had an additional band near the centromere. The results, however, were not sufficient to permit a detailed karyotype analysis, as had been hoped.

CHAPTER 6

DISCUSSION

6.1 Direction of evolution of groups of species within the genus *Vulpia* and *F. rubra* agg.

Investigations into the course of evolution in many different plant genera have shown that as a rule plants with higher ploidy levels have evolved from those of lower ploidy levels, annuals have evolved from perennials, and inbreeders have evolved from outbreeders. These trends have been shown to be reversed in only a relatively few cases. A survey of ploidy level, habit and breeding system of existing species reveals that the combinations of these characters has in some degree determined the way they have evolved. Polyploidy in perennial herbs appears to be just as common in self-fertilising as in cross-fertilising species, but is rare among long-lived plants in which vegetative reproduction is missing. Polyploidy in annuals is almost entirely confined to groups which have a high proportion of self-fertilisation (Stebbins, 1971). Stebbins' (1971) explanation of the correlation between polyploidy and efficient vegetative reproduction in perennial herbs is that new polyploids pass through a "bottleneck" of semi-sterility and that they are much better equipped to do this if they are perennials which can spread by means of vegetative reproduction. Grant (1956) suggests that the commonest process by which chromosome numbers are doubled in short-lived species is by the union of unreduced gametes and, consequently, polyploidy may be expected to become established more frequently in inbreeders than in outbreeders.

Both inbreeding and outbreeding species are found in the genus *Vulpia*; species in sections *Vulpia* and *Monachne* are self-fertile, while those species in section *Loretia* have a high degree of self-sterility,

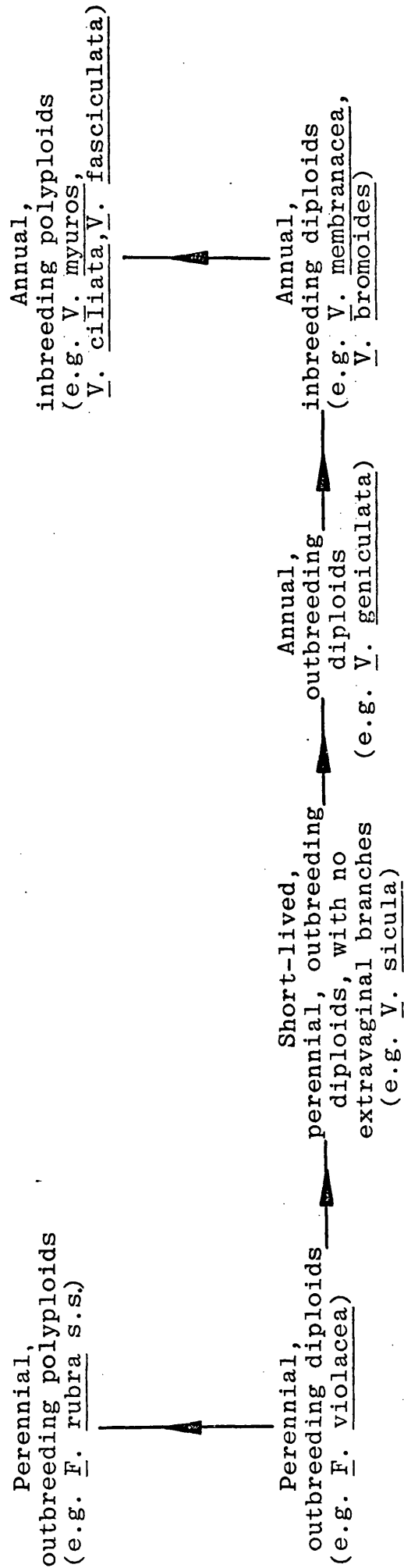
except for one collection of V. alopecuroides which was over 50% self-fertile. Further tests of self-fertility of a range of populations of V. alopecuroides are needed. F. rubra is also largely self-sterile and the results recorded in the present study agree with those of Auquier (1977). Auquier (1977) tested the self-fertility of different subspecies of F. rubra and found that, although there was quite a large variation between different populations, all the subspecies were largely self-sterile.

Of the annual inbreeding species of Vulpia, five species are diploid and seven are polyploid (two tetraploids and five hexaploids). All the annual outbreeding species of Vulpia are diploid, and the outbreeding perennial V. sicula, which has only intravaginal branches, is also diploid. All the species of the F. rubra agg. are perennials and F. rubra and F. juncifolia are outbreeding and polyploid, though four species in the aggregate have a diploid chromosome number. Thus in the genus Vulpia and the F. rubra agg. polyploids are common among inbreeding annuals and in perennials but are absent among the outbreeding annuals.

Fig. 24 shows the probable direction of evolution in the genus Vulpia and the F. rubra agg. Species representing all these steps in evolution exist today. The horizontal pathway in Fig. 24, in which changes of habit and breeding system occurred, was probably the result of gradual speciation. One other distinct perennial species of Vulpia is known, V. litardiereana, which is a densely tufted plant. It is restricted to the Grand Atlas Mountains of Morocco. Unfortunately the chromosome number of V. litardiereana is unknown. The vertical pathways in Fig. 24 represent those arising as a result of abrupt speciation, i.e. with a change in ploidy level.

Using Stebbins' guide to the maturity of polyploid complexes (Stebbins, 1971), the genus Vulpia is an example of a young polyploid complex. In the West Mediterranean region, the primary centre of diversity of species of Vulpia (Cotton & Stace, 1976), ten species are diploids and

Fig. 24 Diagram of the probable direction of evolution in the genus Vulpia and the F. rubra aggregate.



Examples are of extant species showing these combinations, not ancestral taxa.

three are polyploids. The geographical ranges of all these species overlap in North Africa except for those of V. inops and V. fontquerana, and the ranges of many species also overlap in S.W. Europe. The hexaploid V. myuros has the widest distribution of all the European species.

Of the species in the F. rubra agg. for which chromosome numbers have been reported (Table 1), four species have a diploid chromosome number, and 17 species have a polyploid number (tetraploids, four species; hexaploids, eleven species; octoploids, four species; decaploid, one species). As mentioned in Chapter 1, the F. rubra agg. is an example of a mature polyploid complex. The geographical ranges of all four diploids are very restricted but that of F. rubra is the most extensive, spreading throughout Europe to Asia. F. heterophylla ($2n = 28,42$), F. trichophylla ($2n = 42$), F. juncifolia ($2n = 56$), F. diffusa ($2n = 42,56$) and F. nigrescens ($2n = 28,42$) are widespread in some parts of Europe but the rest of the species in the aggregate have a fairly restricted distribution in Europe.

The very small number of present day diploid species in the F. rubra agg. makes it practically impossible to unravel the paths of evolution of the polyploid species. However, from the relatively large number of existing diploid species of Vulpia, the possible ancestral origins of the polyploid Vulpia species may be discovered. An attempt has been made in the present study, using hybridisation, chromosome pairing, phenotypic data and geographical distribution to reveal the relationships between the diploid species of Vulpia and to discover the possible ancestors of a particular polyploid Vulpia species.

6.2 Experimental technique

In all the hybridisation experiments of the present study the germination success was artificially improved by the use of embryo

TABLE 40 Comparison of percentage germination of caryopses with and without embryo culture

	% germination without embryo culture (Cotton)	% germination with embryo culture (Barker)
V. fasciculata x V. geniculata	2%	27%
V. fasciculata x V. alopecuroides	0%	100%
V. fasciculata x F. rubra	100%	85%

TABLE 41 Percentage of caryopses obtained from reciprocal crosses where one parental species is self-compatible and the other is self-compatible.

		% of caryopses obtained from the florets pollinated	
SI x SC		SC x SI	
<u>Barker's (1980) results:</u>			
V. alopecuroides x V. membranacea	2%	Reciprocal	22%
V. geniculata x V. membranacea	0%	Reciprocal	16%
V. ligustica x V. membranacea	0%	Reciprocal	19%
V. sicularis x V. membranacea	0%	Reciprocal	4%
V. alopecuroides x V. fasciculata	0%	Reciprocal	1%
<u>Cotton's (1974) results:</u>			
V. geniculata x V. fasciculata	0%	Reciprocal	39%
V. alopecuroides x V. fasciculata	0%	Reciprocal	2%
F. rubra x V. fasciculata	1%	Reciprocal	43%

culture. Cotton's (1974) attempts at artificial hybridisation between Vulpia species and between species of Vulpia and F. rubra were often successful in producing caryopses but most of the caryopses failed to germinate, except for the V. fasciculata x F. rubra cross, which is also found in the wild.

A comparison of the percentage germination for Cotton's (1974) results and those duplicated in the present study using embryo culture are given in Table 40. The reduction in percentage germination with embryo culture for V. fasciculata x F. rubra was probably due either to infection of the agar or to damage to the embryo during dissection. The use of embryo culture clearly improved the germination of hybrid caryopses which had little or no ability to germinate normally.

6.3 Criteria used to determine relationships

In the present study crossability, performance of the F_1 hybrids, genome pairing and pollen sterility were employed to assess the closeness of relationships between species.

Crossability alone is not a good indication of taxonomic distance between species of Vulpia and F. rubra agg. Since pollen incompatibility and differences in ploidy level can complicate the situation.

Attempts at artificial hybridisation show that a range of crossability exists. In some cases no caryopses were produced at all; in other crosses caryopses were produced but did not germinate with embryo culture; some caryopses had abnormal endosperm but did germinate with embryo culture; and others had near normal endosperm and also gave rise to hybrid plants (Figs. 22 & 23).

The absence of hybrid caryopsis formation in the four interspecific reciprocal crosses (Fig. 22) could have been due to unfavourable conditions in the greenhouse or to faulty technique, but this is thought

FIGURE 22. NATURAL AND ARTIFICIAL HYBRIDISATIONS.

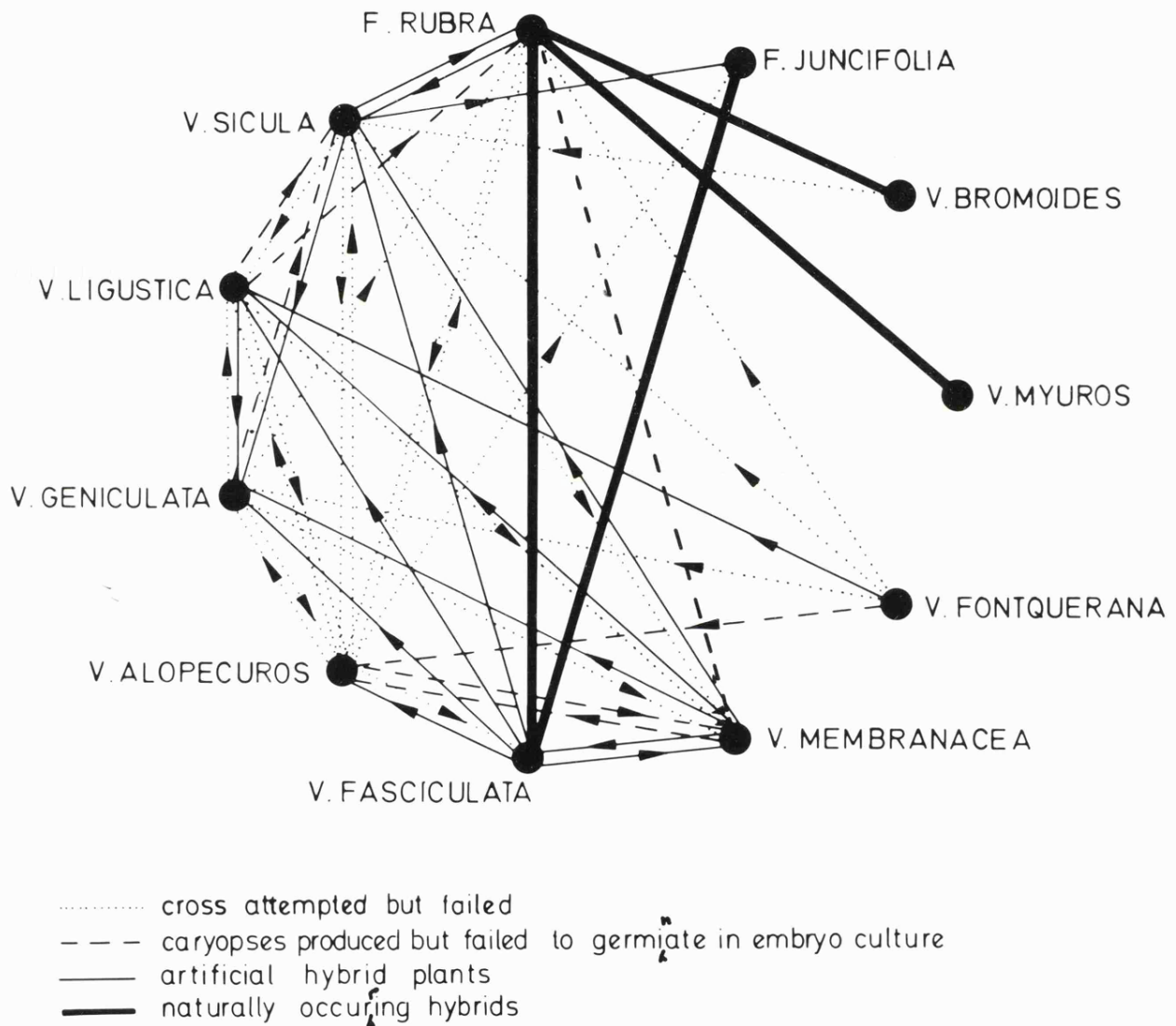
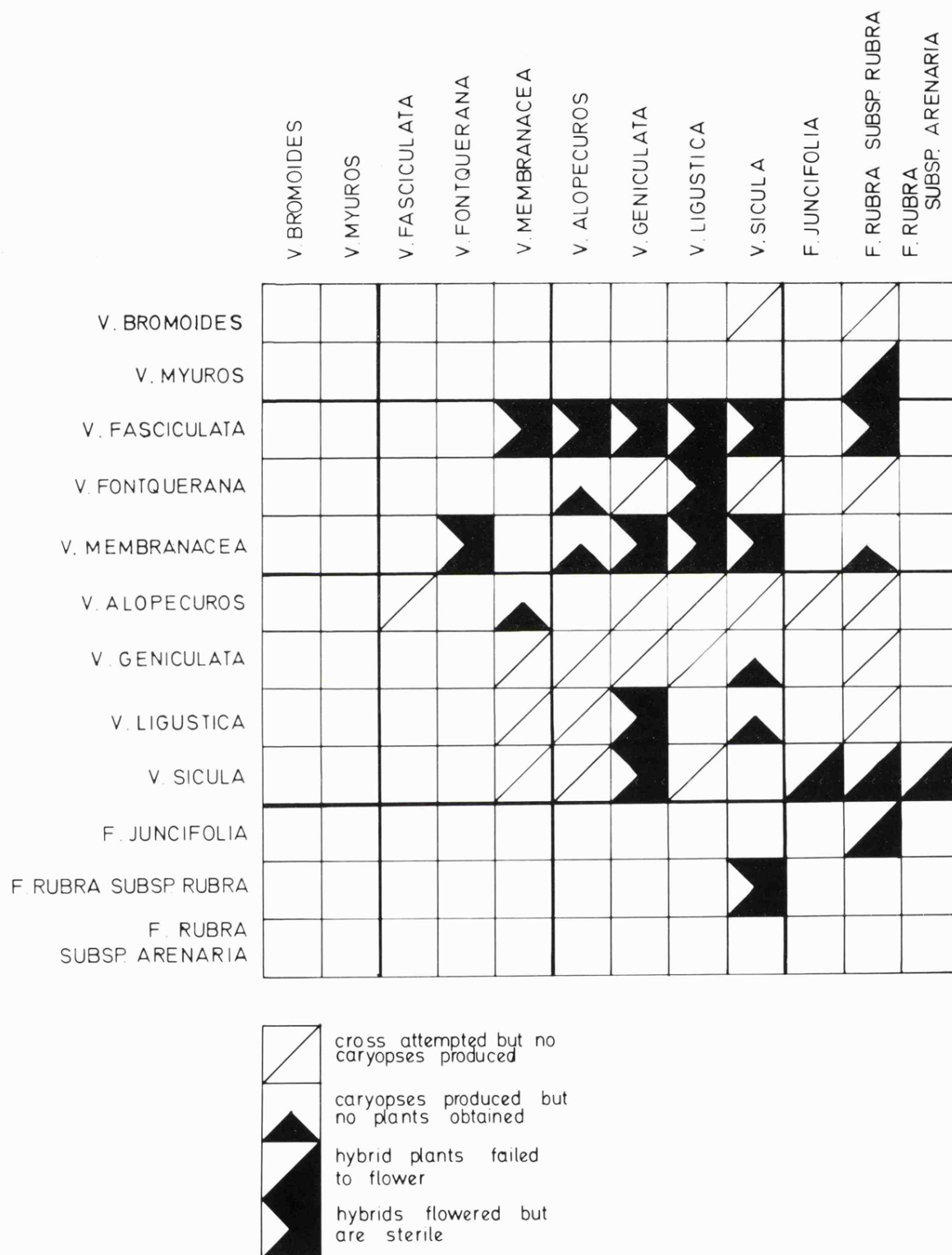


FIGURE 23. RECIPROCAL CROSSABILITY OF SPECIES COMBINATIONS



to be unlikely as the pollinations were repeated over several days and other crosses attempted at the same time were successful. Some of the ovaries which had been cross-pollinated were examined after about 14 days and no growth of the ovary had occurred. The most probable explanation for the failure of these crosses is the presence of pollen incompatibility. Incompatibility in grasses is gametophytic but the germination of the pollen is inhibited by the stigma (Heslop-Harrison, 1978). The occurrence of pollen tube inhibition on the stigmas could be tested.

Caryopses were formed but did not germinate even with embryo culture in two interspecific and two intergeneric crosses (Fig. 22). Lack of germination could have been due to damage to the caryopses during dissection, or to the abnormal endosperm preventing proper development of the embryo, or to incompatibility between parental genomes in the zygote.

Those hybrids obtained from caryopses with abnormal endosperm (Table 8) are very unlikely to occur in nature. The abnormal endosperm could have resulted from either the endosperm being female only or the parental genetic material being incompatible and unable to produce endosperm at all. On the other hand, those caryopses with hard, near normal endosperm which produced hybrids after embryo culture may well have been able to germinate without embryo culture. V. fasciculata x F. rubra hybrids occur naturally and Cotton (1974) obtained one hybrid plant, V. fasciculata x V. geniculata, without embryo culture.

Thus the crossability of these species is prevented at three stages. The internal breeding barriers are probably pollen incompatibility (gametophytic isolation), incompatibility of genetic material (gametic isolation) and abnormal endosperm preventing germination (seed incompatibility).

The caryopses from both interspecific and intergeneric crosses with at least one parent at a higher ploidy level tended to have a less deficient endosperm and a better germination than caryopses from diploid-diploid crosses. The embryo depends on food supplies from the endosperm during its development and when the endosperm is deficient the germination success is lower than those caryopses with a more normal endosperm.

The interspecific Vulpia diploid and triploid hybrids which produced caryopses showed no reciprocal differences in embryo or endosperm types and the percentage germination of the reciprocal hybrids was also very similar. However, the hexaploid-diploid F. rubra x V. sicula intergeneric cross did differ from its reciprocal for embryo size and germination (Table 8). Valentine and Woodell (1963) hybridised various species of Primula and found that reciprocal crosses between diploids and autotetraploids consistently produced different types of F_1 seed. They also found the same two types, but to a lesser degree, in reciprocal diploid crosses. Gymer and Whittington (1973) hybridised the diploids Lolium perenne and Festuca pratensis. When L. perenne was used as the female parent, small seeds with solid endosperm were produced and from the reciprocal cross, large seeds with a watery endosperm were produced which were less viable than the smaller seeds. Similar reciprocal differences occurred in diploid-autotetraploid crosses: diploid-tetraploid crosses gave large, watery seeds and tetraploid-diploid crosses gave small, fat seeds.

Crossability is also unreliable as a criterion in this group of species unless reciprocal crosses are attempted, as unilateral incompatibility exists in many crosses. Table 41 lists the percentage of caryopses obtained for reciprocal crosses where one parental species is self-incompatible and the other is self-compatible, both in this research

project and that of Cotton (1974). It can be seen from Table 41 that when self-incompatible species (female) are pollinated by self-compatible species practically no caryopses are produced whereas the reciprocal crosses are much more successful.

In the present study reciprocal differences in crossability were also found between the following self-incompatible species (percentage of caryopses obtained from florets pollinated): V. geniculata x V. ligustica (0%), reciprocal (67%); V. geniculata x V. sicula (0.7%), reciprocal (26%). Cotton (1974) also found the following intergeneric cross with reciprocal differences: V. geniculata x F. rubra (0%), reciprocal (85%). In all the crosses between self-incompatible species, when V. geniculata was used as the female parent the cross was unsuccessful.

Unilateral interspecific incompatibility is a phenomenon found in many different plant genera (de Nettancourt, 1977), and it is not always restricted to SI x SC relationships, but has been observed between self-incompatible species in other genera. It has been proposed by Grun and Radlow (1961) and Abdalla (1970) that unilateral incompatibility has developed in response to the possibility of hybridisation between self-incompatible and self-compatible populations in order to keep the character of self-compatibility away from the self-incompatible populations, which would otherwise lead to inbreeding depression and uncovering of recessive lethals in erstwhile self-incompatible populations.

An alternative explanation of unilateral incompatibility is that given by Watkins (1932) concerning the ploidy ratio of pollen: style, but this hypothesis is not upheld by the results of the present study.

The performance of the F_1 hybrids (Table 30) does seem to be correlated with the taxonomic distance of the cross. Four interspecific Vulpia crosses produced hybrids which took several months longer to produce inflorescences than the other interspecific hybrids, which produced only a

few inflorescences and had poor vegetative growth. Of the interspecific Vulpia crosses which produced vigorous hybrids, three crosses were between species in the same taxonomic section and four crosses were between species in different sections, whereas all the less vigorous hybrids were between species in different sections.

Analysis of bivalent pairing at meiosis in the parental species and their hybrids is recognised as a means of determining the closeness of relationship between parental genomes. An analysis of pairing in diploid hybrids is straight-forward as there are two genomes and the amount of pairing between these genomes directly shows how similar are the parental genomes. However, deductions from pairing in triploid hybrids and those of higher ploidy levels are not as straight-forward, especially if the chromosomes have no distinguishing features. The large range in number of bivalents observed in all the artificial hybrids may be explained by desynapsis.

Of the diploid hybrids, V. ligustica x V. geniculata has the highest number of bivalents and highest chiasma frequency (Table 31). Both values are similar to those found in the parental species and this suggests a close similarity between the genomes of V. ligustica and V. geniculata. The other four diploid hybrids had fewer bivalents and lower chiasma frequencies. Apparent trivalents were very occasionally seen in two of the diploid hybrids, though this was possibly due to the slide preparation with chromosomes slightly overlapping each other. V. ligustica and V. geniculata both occur in the same section, Loretia, and are very similar morphologically. The parental species of the other diploid hybrids occur in different sections, so that the amount of pairing in the diploid hybrids does seem to be correlated with the taxonomic distance of the cross.

All the triploid hybrids had some cells with eight or nine

bivalents. The presence of more than seven bivalents may possibly have been an artefact. But if not, then there must have been some intra-genomic pairing. In all the triploid hybrids, some cells were observed with trivalents (Table 31). V. membranacea x V. fasciculata had the largest number of trivalents; 0 to 3 trivalents were found but over half the cells had no trivalents and very few had three. Therefore there was no set pattern of occurrence of trivalents and it is difficult to establish whether or not the trivalents were formed because the genomes had a reciprocally translocated segment. The range of bivalents in the triploid hybrids was wide (Table 31), but the means ranged from 5.8 to 6.3 (Table 31). Either heterogenetic pairing or homogenetic pairing between the two V. fasciculata genomes could have been occurring in the triploid hybrids.

The F. rubra x V. sicula hybrids had a mean of 8.18 bivalents and no multivalents. There must have been some pairing between two of the F. rubra genomes and between one F. rubra genome and the V. sicula genome. However it is not known how many bivalents of each type were produced.

The chromosomes forming bivalents (Table 31) in the F. rubra x V. fasciculata and F. juncifolia x V. fasciculata crosses are of unknown parental origin because of the possibility of both heterogenetic and homogenetic pairing, though in the case of F. juncifolia x V. fasciculata there must have been some homogenetic pairing (between F. juncifolia genomes).

All the artificial hybrids except V. ligustica x V. geniculata and V. fasciculata x F. rubra had 100% sterile pollen and the two exceptions had only 2.0 - 2.5% stainable pollen. The natural V. fasciculata x F. rubra hybrids also had very low amounts of stainable pollen but a single F₂ plant was obtained from one hybrid. The complete or very high

pollen sterility in all the hybrids, due to irregular meiosis or to inviable pollen grains as a result of cryptic structural hybridity, does not reveal differences in taxonomic distance between particular pairs of species.

6.4 Relationships between the diploid species of *Vulpia*

Eight Mediterranean diploid species of *Vulpia* occur in the W. Mediterranean region, the primary centre of diversity. The present day distributions of the diploid species of *Vulpia* are largely sympatric, particularly in North Africa. There are no exclusive habitat preferences for species except for *V. litardiereana*, which occurs at high altitudes of 2,400 - 2,800 metres (Maire, 1955), in the Grand Atlas, *V. sicula*, which occurs only in closed turf, and *V. alopecuroides*, which occurs on maritime sands as well as the dry, open habitats preferred by the other species. All the interspecific hybrids examined in the present study were sterile; thus the parents are good biological as well as distinct morphological species.

The present study has revealed a close relationship between *V. ligustica* and *V. geniculata*, although cryptic structural differences between these two genomes prevent much viable pollen being formed in the hybrid. The relationship between *V. sicula* and *V. geniculata* is also thought to be close due to the performance of the F_1 hybrid (Table 30), though meiotic observations are lacking for this hybrid. *V. sicula* and *V. ligustica* are probably prevented from pairing in reciprocal crosses by pollen incompatibility barriers. These two species are sympatric over all of their distributions except where *V. ligustica* extends further east than *V. sicula*. It is proposed that a pollen incompatibility barrier has developed naturally between these species and this prevents them from crossing and that in fact they are probably very closely related. Therefore there is some evidence that these three species of *Loretia* are quite

closely related. V. alopecuroides is also classified as a species of section Loretia (Cotton & Stace, 1977) due to its chasmogamous florets and large anthers. However, V. alopecuroides does differ obviously from the other species of section Loretia in that it has a glabrous ovary, a long, pointed callus, and long hairs on the edges of the lemma. Attempts at crossing V. alopecuroides with the other species of section Loretia proved totally unsuccessful and no caryopses at all were formed in reciprocal crosses (Fig. 23); this suggests the presence of strong incompatibility barriers. The lack of success in crossing the diploid species of Monachne with V. alopecuroides is likely to be a result of genetic incompatibility as hybrid caryopses were produced but did not germinate. Because the diploid species of Monachne crossed easily with V. sicula, V. geniculata and V. ligustica but not with V. alopecuroides (Fig. 23), it is proposed that whereas V. sicula, V. geniculata and V. ligustica seem to be a closely related group of species, V. alopecuroides is very different from them. V. alopecuroides in fact has some morphological similarities to species of section Monachne, but the inability of V. alopecuroides to hybridise with the diploid species suggests also many genetic differences.

The unilateral interspecific incompatibility between the self-incompatible species V. geniculata and V. ligustica and between V. geniculata and V. sicula obviously reduces the possibility of hybridisation in one direction, but the underlying mechanism of the barrier being unidirectional is not clear.

Of all the European species of Vulpia, V. fontquerana is the only species that does not occur in North Africa. V. fontquerana is restricted to a very small area in S. Spain and is considered by Cotton and Stace (1976) to be a relict species. Crossing between V. fontquerana and V. membranacea (section Monachne) was not attempted, but they are presumed to be closely related as they have many morphological similarities.

Of the Monachne-Loretia hybrids, V. membranacea and V. geniculata appear to have the closest relationship from the chromosome pairing results (Table 31) and the performance of the F_1 hybrid (Table 30). The other hybrids, V. membranacea x V. ligustica, V. membranacea x V. sicula and V. fontquerana x V. ligustica, all had fewer bivalents at meiosis, delayed production of inflorescences, reduced vegetative growth and fewer inflorescences (Table 30). The diploid, European species of section Vulpia, V. bromoides and V. muralis, were not crossed with any other diploid species of Vulpia due to the practical difficulties involved.

The possibility of sympatric speciation in cross-fertilising species has been much debated (Grant, 1971). Conclusive evidence supporting sympatric speciation in flowering plants is lacking, and Mayr (1963) others regard sympatric speciation as unlikely. A more likely explanation for the speciation of the diploid, outbreeding species of Vulpia is the build up of breeding barriers between divergent peripheral populations and then the spread of these new species back into the central area, i.e. North Africa. The evolution of semi-cleistogamy, i.e. in V. fontquerana and V. membranacea, would restrict gene exchange between these and other types and, as a result of divergence, additional breeding barriers might have developed. Further changes to complete cleistogamy, i.e. in V. bromoides and V. muralis, resulting in inbreeding, has allowed these types to become pioneer species and as a result the distributions of species of section Vulpia are far more extensive than those of other sections.

6.5 The possible ancestors of *V. fasciculata*

V. fasciculata is a tetraploid which forms only bivalents at meiosis. *V. fasciculata* is very similar morphologically to *V. membranacea* and sometimes the two species can only be separated by the presence or absence of pubescence on the ovary tip. Thus *V. membranacea* seems to be a likely donor of one, or both, genomes if *V. fasciculata* is an autopolyploid. *V. fontquerana* and *V. alopecuros* also have morphological similarities to *V. fasciculata*.

If *V. fasciculata* were a fairly recent autopolyploid from *V. membranacea* or one of the other diploid species of *Vulpia*, a high number of trivalents would be expected in the relevant hybrid, but this was not found to be true for the artificial hybrids (Table 31).

If then it is assumed that *V. fasciculata* is an allopolyploid and pairing in the triploid hybrids is heterogenetic, then in a triploid hybrid with about seven bivalents, the genome of the diploid parent would be similar to one genome of *V. fasciculata*. However, in all the artificial triploid hybrids, from *V. membranacea* to *V. sicula* (obviously very different from *V. fasciculata*) as parental species, means of 5.3 to 6.3 bivalents and low chiasma frequencies occurred (Table 31). It is possible that in the five triploid hybrids, different genomes of *V. fasciculata* were forming bivalents with the genomes of the different diploid parental species. Unfortunately, as the chromosome complements of all species of *Vulpia* are very similar, the different genomes cannot be recognised at meiosis.

V. fasciculata has a widespread distribution; it reaches further north than *V. membranacea* and much further north than any species of section *Loretia*. The origin of the pubescent tip to the ovary in *V. fasciculata* from present day species would be any of the species from section *Loretia* except *V. alopecuros*, although these species are very

dissimilar from V. fasciculata in other morphological characters.

If V. fasciculata is an autopolyploid it is possible that a regulator gene is present, as in wheat (Riley & Chapman, 1958), to prevent multivalent formation. Multivalents (a range of one to seven quadrivalents) were observed in the autotetraploid from colchicined V. geniculata.

In the V. membranacea x V. fasciculata hybrids the callus length was found to be slightly longer than in either parental species (Table 32). This is exceptional as all other inflorescence characters, except anther length, were intermediate between the range of that of the parental species. V. fontquerana has a longer callus than both V. membranacea and V. fasciculata (and their hybrids).

6.6 The relationship between the genus Vulpia and F. rubra agg.

Although the species of Vulpia and the F. rubra agg. have undergone many changes during their evolution from the original common ancestors, their capacity for hybridisation has not been eliminated. Of the four natural hybrids, F. rubra x V. fasciculata has been found most often. V. fasciculata x F. rubra hybrids have been reproduced artificially both by Cotton (1974) and in the present study. Cotton (1974) found that the cross could only be made with V. fasciculata as the female parent. Nothing can be deduced of the relationships between the parental genomes from the meiotic results in the natural or artificial V. fasciculata x F. rubra hybrids.

The V. myuros x F. rubra hybrid has been reproduced artificially in the present study. The V. myuros x F. rubra artificial hybrid failed to produce inflorescences in two summers and this behaviour was similar to that of the natural V. myuros x F. rubra hybrid and the natural V. bromoides x F. rubra hybrid growing at Leicester, which have produced

inflorescences in some years and not in others. Shy flowering is possibly the reason for the failure to find V. bromoides x F. rubra hybrids in the wild in 1974 and 1975, despite their presence in 1969, 1973 and 1976 at the same locality. Populations of V. bromoides and V. myuros have been found with chasmogamous florets (Auquier & Stace, 1980) and it is probable that V. bromoides and V. myuros were the female parents of the wild hybrids with F. rubra.

The natural V. bromoides x F. rubra hybrid had the unexpected chromosome number of $2n = 42$ or 43 . The simplest explanations for this chromosome number are either that the F. rubra parent had a chromosome number of $2n = 56$ and the female parent V. bromoides produced unreduced gametes, or that a hybrid between octoploid F. rubra and diploid V. bromoides produced a pentaploid hybrid ($2n = 35$) and this backcrossed to the octoploid F. rubra parent to produce F_2 hybrids with about 45 or fewer chromosomes. If the latter was the case, then through backcrossing the hybrid to the F. rubra parent, 28 bivalents plus a few univalents would be expected. If the former was the case then seven bivalents would be expected from the unreduced V. bromoides gamete and possibly any other bivalents from pairing between F. rubra genomes (Chapter 6). A range of five to 14 bivalents was observed in the V. bromoides x F. rubra hybrid (Table 34) and thus the backcrossed origin of this hybrid is ruled out. The non-reduced V. bromoides origin becomes a plausible explanation, with, in this case, a very small degree of pairing between F. rubra genomes. However, evidence of pairing between the genomes of the two different genera in this hybrid is lacking.

The perennial species V. sicula and V. litardiereana seem to be the obvious link species between the perennial fescues and the annual species of Vulpia (Fig. 24). Artificial hybrids were made between V. sicula and three species of Vulpia, V. geniculata,

V. membranacea and V. fasciculata. In the V. membranacea x V. sicula hybrids a mean of 4.5 bivalents were formed and therefore these two species do have some similarities in their genomes, although the chiasma frequencies (Table 31) were low. It has already been stated that V. sicula is believed to be very closely related to V. ligustica and V. geniculata.

In the F. rubra x V. sicula hybrids the high number of bivalents (mean = 8.2) suggests that there must have been some heterogenetic pairing between the genomes of V. sicula and F. rubra as well as homogenetic pairing between two F. rubra genomes. Two to five ring bivalents were observed at meiosis in the hybrids but the number of bivalents involved in the two types of pairing is unknown and the relative amounts of rod and ring bivalents for either type of pairing is therefore also unknown. However, V. sicula does have a genome or part of a genome similar to one of the F. rubra genomes, so these species probably had a common ancestral type.

6.7 Origins of the polyploids F. rubra and F. juncifolia

F. rubra and F. juncifolia have 21 and 28 bivalents respectively at meiosis. These polyploids must either be amphidiploids with only homogenetic pairing occurring at meiosis or have a regulator gene, as is found in Triticum, to prevent multivalent formation at meiosis. As has already been concluded from evidence presented in the present study, some preferential pairing between two F. rubra genomes can occur in some of the hybrids. Homogenetic pairing between F. rubra genomes must have occurred in F. rubra x V. sicula hybrids and probably in the V. bromoides x F. rubra ($2n = c.42$), and pairing between F. juncifolia genomes must have occurred in the V. fasciculata x F. juncifolia hybrid. Thus in both F. rubra and F. juncifolia it is

likely that there is a regulator gene which prevents multivalent formation at meiosis. In none of these hybrids was a high number of trivalents or quadrivalents observed. It can therefore be deduced that the three genomes of F. rubra or the four genomes of F. juncifolia are not all similar.

Jauhar (1975) found that in hybrids between Lolium perenne ($2n = 14$) and F. rubra ($2n = 42$), there was a range of ten to 14 bivalents with a mean of 12.1 bivalents and very few multivalents, and a high mean chiasma frequency of 20.2 per cell was observed. This suggests that there was both homogenetic pairing between the F. rubra genomes and heterogenetic pairing between one F. rubra genome and the L. perenne genome. This supports the conclusion drawn from the present study that two F. rubra genomes can pair together and that there is a regulator gene operating at meiosis in F. rubra. However, this also suggests that one genome of F. rubra is similar to that of L. perenne and the present study indicates a similarity between ~~one~~ genome and that of V. sicula.

6.8 Intergeneric hybrids as possible contributors to the variability of F. rubra.

The only hybrid from which seed was obtained was a natural clone of V. fasciculata x F. rubra. This was found to be approximately 0.006% fertile, but the single F_2 plant obtained has not yet produced inflorescences. It is an approximate hexaploid ($2n = 41,45$), and thus this hybrid combination could potentially contribute to the variability of F. rubra. An example of variability being introduced into a species by a hybrid is given by Jones and Carroll (1962) for the origin of the pentaploid chromosome race of Holcus mollis. H. mollis occurs in Britain as a complex of polyploids ($2n = 28,35,42,49$) and Jones (1958) proposed the hybrid origin of H. mollis from H. lanatus. A greater

variability is found in many characters of the pentaploid when compared with other races, but pentaploid H. mollis is easily mistaken for pure H. mollis and is often classified as such.

If backcrossing of the V. bromoides x F. rubra hybrid ($2n = 42$) to F. rubra occurred naturally this could also add to the variability of F. rubra.

6.9 Further investigations

The use of embryo culture in this project has allowed 20 different hybrid combinations to be produced, and therefore studied, most of which would otherwise not have been produced if the hybrid caryopses were allowed to germinate normally. It is possible that spraying the pollinated ovaries with gibberellic acid and removing the embryos for culture 10 to 14 days after pollination (as described in Chapter 2) could increase both the number of hybrid plants and the number of species combinations obtained.

The analysis of genome pairing in triploids and hybrids of high ploidy levels proved very confused and very few conclusions could be made. However, the analysis of genome pairing in hybrids between diploid species immediately revealed species relationships. Unfortunately the diploid species of section Vulpia were hardly used in the crossing programme because of the practical difficulties involved and it may be profitable to improve techniques of emasculation and pollen collection in these cleistogamous species. It is possible, however, that seed protein electrophoresis, as used in wheat (Johnson & Hall, 1965), or serological techniques, as used in Bromus (Smith, 1971; 1972), may prove to be a more straight-forward way of elucidating the relationships between the diploid species of section Vulpia and other diploid species of Vulpia.

The ancestral origin of tetraploid V. fasciculata remains uncertain but the type of pairing occurring in the triploid hybrids could

be determined by making autotetraploids of the diploid species of Vulpia and crossing an autotetraploid with V. fasciculata. If still only bivalents were formed, it would confirm that V. fasciculata genomes were pairing together. If, however, up to seven trivalents were formed then the pairing must be occurring between one V. fasciculata genome and the genome of the diploid parent. If Giemsa staining techniques were developed and could be used at meiosis in the triploid hybrids, this may be another way of determining the type of pairing occurring in these hybrids.

APPENDIX

Specimens of <i>Vulpia</i> grown from seed of wild origin			Chromosome number (2n)
V 5	<i>V. membranacea</i>	Portugal	14
V 30	<i>V. alopecuroides</i>	Sanlúcar de Barrameda, Cádiz, Spain	14
V 33	<i>V. alopecuroides</i>	Coimbra, Portugal	14
V 36	<i>V. membranacea</i>	Coimbra, Portugal	14
V 65	<i>V. membranacea</i>	Normandy, France	14
V 79	<i>V. alopecuroides</i>	Lisbon, Portugal	14
V 82	<i>V. geniculata</i>	Lisbon, Portugal	14
V 110	<i>V. bromoides</i>	Sandwich, Kent, v.c. 15	14
V 136	<i>V. bromoides</i>	Shingle Street, Suffolk, v.c. 25	14
V 181	<i>V. membranacea</i>	Algaida, Cádiz, Spain	14
V 198	<i>V. myuros</i>	Burton-on-Trent, Staff., v.c. 39	42
V 240	<i>V. alopecuroides</i>	Coimbra, Beira Litoral, Portugal	14
V 254	<i>V. ligustica</i>	S. Nicola, N.W. of Messina, Sicily	14
V 255	<i>V. ligustica</i>	La Pizzuta, Piana degli Albanesi, Sicily	14
V 257	<i>V. ligustica</i>	M. Cuccio, Torretta, S.W. of Palermo, Sicily	14
V 260	<i>V. fasciculata</i>	Spadafora, Milaggo, Sicily	28
V 263	<i>V. fasciculata</i>	M. Cuccio, Torretta, S.W. of Palermo, Sicily	28
V 294	<i>V. fasciculata</i>	Berrow, Somerset, v.c. 6	28
V 303	<i>V. bromoides</i>	Swansea, Glamorgan, v.c. 6	14
V 356	<i>V. geniculata</i>	Coimbra, Beira Litoral, Portugal	14
V 364	<i>V. membranacea</i>	S.W. of Turrillas, Almería, Spain	14
V 365	<i>V. sicula</i>	Ficuzza, Sicily	14
V 404	<i>V. fontquerana</i>	Algaida, Sanlúcar de Barrameda, Cádiz, Spain	14
V 423	<i>V. membranacea</i>	Étaples, Pas de Calais, France	14

Specimens of <i>Festuca</i> grown from seed of wild origin			Chromosome number (2n)
F 3	<i>F. juncifolia</i>	Littlehampton, Sussex, v.c. 13	56
F 6	<i>F. rubra</i>	Harlech, Merioneth, v.c. 48	42
F 7	<i>F. rubra</i>	Barmouth Junction, Merioneth, v.c. 48	42
F 11	<i>F. rubra</i>	Braunton, Devon, v.c. 4	42
F 21A	<i>F. rubra</i> subsp. <i>arenaria</i>	Le Touquet, Pas de Calais, France	56
F 21B	<i>F. juncifolia</i>	Le Touquet, Pas de Calais, France	56
F 31	<i>F. rubra</i>	Dunbeath, Caithness, v.c. 109	42
F 35	<i>F. rubra</i>	Montreuil, Pas de Calais, France	42
F 37	<i>F. rubra</i>	Berrow, Somerset, v.c. 6	42
F 39	<i>F. rubra</i>	Lesmahagow, Lanarkshire, v.c. 77	56
F 41	<i>F. juncifolia</i>	Thornham, W. Norfolk, v.c. 28	56
F 43	<i>F. rubra</i>	Higher Poynton, Cheshire, v.c. 58	42
F 44	<i>F. rubra</i>	Manchester, Lancashire	42
F 45	<i>F. rubra</i>	Stockport, Cheshire, v.c. 58	42
F 51	<i>F. rubra</i> subsp. <i>litoralis</i>	Mont St. Michel, Manche, France	
F 70	<i>F. rubra</i> subsp. <i>commutata</i>	Angleur, Liège, Belgium	
F 71	<i>F. rubra</i> subsp. <i>planifolia</i>	Baden, Liège, Belgium	
F 89	<i>F. rubra</i>	Brittany, France	
F 90	<i>F. rubra</i>	Brittany, France	

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ABSTRACT

Investigation into the relationships and ancestry of Vulpia and Festuca

C.M. Barker

The relationships between the European species of Vulpia were investigated by interspecific hybridisation and consequent meiotic analysis, hybrid fertility and morphological survey of the F_1 hybrids. The relationship between the genus Vulpia and the Festuca rubra aggregate was investigated by the examination of both naturally occurring and artificially produced intergeneric hybrids.

Embryo culture was used for both interspecific and intergeneric crosses and the germination of the hybrid embryos by this method proved successful. Of 47 cross combinations (including reciprocals) attempted hybrid plants were obtained for 20 different crosses.

Crossability and hybrid fertility were not found to be obvious indicators of taxonomic distance and unilateral incompatibility was found both in crosses with a self-incompatible species as the female parent and a self-compatible species as the male parent and in crosses where both parental species were self-compatible.

Analysis of bivalent pairing at meiosis revealed genome relationships in hybrids between diploid species. However no conclusions could be made as to the possible ancestors of tetraploid V. fasciculata.

Artificial hybrids were made between V. sicula, the presumed link species between the annual species of Vulpia and the perennial fescues, and F. rubra and the meiotic analysis of the F_1 hybrid revealed similarities between at least part of the V. sicula genome and a genome of F. rubra. Conclusions as to genome homology could not be made from the cytological analysis of the other intergeneric hybrids.