Biosystematics of some species of epilobium

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BIOSYSTEMATICS OF SOME SPECIES OF EPILOBIUM

BY

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M.Sc. (PATNA)

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BIOSYSTEMATICS OF SOME SPECIES OF EPILOBIUM

V. Thakur

Abstract

Most European species of Epilobium are known to hybridize, and many occupy overlapping habitats. All have the same chromosome number ($2n = 36$) and their chromosomes are similar. The main aims of the thesis have been to inquire into isolating mechanisms in the genus and to study systematic relationships between its members. 16 species have been used, 14 European and two from New Zealand. Herbarium records indicate that, in Britain, interspecific hybrids are relatively rare.

Internal isolating mechanisms which have been studied include (a) slow growth of pollen-tubes in hybrid crosses, (b) post-fertilization failure of the hybrid seed, (c) failure of hybrid seed to germinate, (d) weakness of hybrids, and (e) sterility of hybrids, both F1 and F2. The fact that many of the species are regularly self-pollinated has also an important bearing on isolation, and helps to account for the relative scarcity of interspecific hybrids in nature as revealed by herbarium studies.

The hybridization experiments have shown that E.angustifolium and E.fleischeri, which belong to the Section Chamaenerion, are a separate breeding group distinct from Sect. Lysimachion. This supports the separation of the two sections of the genus made on morphological grounds. The view that Chamaenerion be treated as a distinct genus is not supported by the evidence derived from the present work. Separation of Lysimachion into two groups, Division Schizostigma and Division Synstigma, on the basis of whether the stigma is 4-lobed or entire, is artificial and is not justified either by hybridization experiments or on morphological grounds. The two New Zealand species do appear to be morphologically distinct from the European species studied ; one of them is able to hybridize with the European species, and the other not.

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CHAPTER 1

INTRODUCTION

Epilobium L. (Onagraceae) is a fairly large genus and is today estimated to have more than 200 species distributed in almost all the continents. The distribution, however, is mainly restricted to the temperate and colder parts of the world.

An excellent taxonomical treatment of the genus has been provided by Haussknecht (1884) in his "*MONOGRAPHIE DER GATTUNG EPILOBIUM*", which still remains the major source of reference for the criteria employed in delimitation and description of the various species. He divides the genus into two sections: Sect. 1. *Chamaenerion* Tausch, and Sect. 11. *Lysimachion* Tausch. Only about 10 to 12 species are included in Chamaenerion and they all form a very distinct group. *Lysimachion* comprises the rest of the species forming a large assemblage of variable plants. The section *Lysimachion* has been subdivided again into Division *Schizostigma* and Division *Synstigma* on the basis of whether the stigmas are lobed or entire.

The generic limits of *Epilobium* have always been questioned. Recently, quite a few taxonomists, especially in Europe, have come to regard *Chamaenerion* Adans. as a separate genus from *Epilobium* proper. Raven (1962a, 1962b) has considered the matter and finds that nothing is to be gained from this separation. There are others who prefer subgeneric rank for the two sections (Rouy and Camus, 1910). However, all that needs to be done at this stage is to point out that a critical gap exists between the two sections in their taxonomical relationship. This relationship has, so far, been assessed differently, providing ample reasons for its re-evaluation.

The world distribution of different species of the genus is very interesting. There are in New Zealand more species than in any other single region of the world, but Sect. Chamaenerion and members of the Schizostigma are entirely missing from there. It is surprising that forms with lobed stigmas should not have arisen in New Zealand. Nearly all of the 32 groups of Lysimachion mentioned in Haussknecht's monograph have a geographical basis, and most of the species are confined to specific centres. Only a few of them have distributions extending from one major geographical region to another (Table 1). E.angustifolium L. seems to be the most widely spread member of the genus and is found in Africa, America, Asia and Europe. E. palustre L. and many members of the group Alpinae are found in Europe, America and Asia. E. hirsutum L. and E. parviflorum Schreb. extend both in Europe and in Asia but are not found in America.

While major geographical isolation on a world basis appears to have resulted in evolution of distinct geographical groups,

TABLE 1

Group-wise distribution of EPILOBIUM in different continents

			ASIA	AMERICA	EUROPE	OCEANIA	AFRICA
CHAMAENERION			+	+	+	−	+
LYSIMACHION	Schizostigma	Chrysonerion	+	+	−	−	−
		Eriophorae	+	−	+	−	+ ◘
		Montanae	+ Δ	−	+	−	+ O
		Stenocalyx	−	+	−	−	−
		Brachycarpae	−	+	−	−	−
		Gayanae	−	−	+	−	−
		Capenses	−	−	−	−	+
	Synstigma	Tetragonae	+ Δ	−	+	−	+
		Chinenses	+	−	−	−	−
		Petiolatae	+	−	+	−	−
		Anadolicae	+	−	−	−	−
		Palustrifoliae	+	−	−	−	−
		Organifoliae	+	+	−	−	−
		Royleanae	+	−	−	−	−
		Brevifoliae	+	−	−	−	−
		Japonicae	+	−	−	−	−
		Palustriformis	+	+	+	−	−
		Himalayenses	+	−	−	−	−
		Nepalenses	+	−	−	−	−
		Platyphyllae	+	+	−	−	−
		Alpinae	+	+	+	−	−
		Glaberrimae	−	+	−	−	−
		Pilosiusculae	−	+	−	−	−
		Tetragonoideae	−	+	−	−	−
		Denticulatae	−	+	−	−	−
		Glaucopides	−	+	−	−	−
		Similes	−	−	−	+	−
		Microphyllae	−	−	−	+	−
		Sparsiflorae	−	−	−	+	−
		Dermatophyllae	−	−	−	+	−
		Schimperinae	−	−	−	−	+
		Anomalifoliae	−	−	−	−	+

Δ Only in Asia Minor. ◘ Only in N. Africa. O E. lanceolatum only.

this factor, when assayed in the context of one region, hardly proves to be of much significance. Confining ourselves mainly to the European species, let us examine specifically the problems of evolution in the genus.

There are about two dozen species in Europe and they belong both to Sect. Chamaenerion and Sect. Lysimachion; the latter is well represented by forms with quadrifid as well as entire stigmas. A recent account has been given by Raven (1964). Cytologically, they are all alike; each species has the same chromosome number, 2n = 36. The chromosomes are very small in size (the details are given in Chapter .V...) and it is hard to find any considerable difference from species to species. This being so, there would seem to have been little change in the cytological make-up of the species during the course of their evolution.

Most of these European species have the same or superimposed ranges of distribution within the continent and thus are sympatric. Not only that, they are often ecologically overlapping. This situation has been discussed by Valentine (1951) in connection with the distribution of E. hirsutum L. and E. parviflorum Schreb. in the British Isles. The habitat of the two species is difficult to distinguish and they have a wide range of distribution throughout Europe. In spite of the fact that they are capable of producing hybrids in nature, they seldom do so, and remain distinct.

Lewis and Moore (1962) have described a similar example, viz. E. adenocaulon Haussk. and E. brevistylum Barbey in the Rocky Mountains. The two species occur together in many localities and are interfertile; but hybrids are rarely encountered in nature and the species remain perfectly distinct. The fact that many species of Epilobium produce natural hybrids is, of course, well established. Many such hybrids have been reported from Europe and according to Clapham, Tutin and Warburg (1962), a high proportion of interspecific hybrids of all combinations are found in the British Isles. Thus, many Epilobium species share the same habitat and are interfertile, yet they remain distinct. What keeps them distinct? What are the isolating factors?

Evolution is dependent upon isolation, and the role of isolation has been described in many publications, e.g., by Dobzhansky (1941), Huxley (1942), Stebbins (1950), and Mayr (1963). In the following paragraphs we shall discuss isolation, from the point of view of the factors which are concerned in the maintenance of taxonomic species, with special reference to Epilobium.

Isolating mechanisms have been classified on the basis of how and at which stage the isolation works. Populations are often geographically isolated; but if they occupy the same area, i.e., are sympatric as opposed to allopatric, the prevention of interbreeding depends upon a variety of their intrinsic or physiological properties, collectively termed as reproductive or physiological isolation. Although geographical isolation would apparently seem to be just a kind of reproductive isolation, many geographically isolated populations have been found to interbreed successfully when brought together. Therefore, the distinction between the two must be borne in mind.

The subdivision of physiological isolating mechanisms can be made in various ways. In whichever way it is made, it is quite safe to put these isolating mechanisms into two distinct categories, (i) external and (ii) internal. External isolating factors work towards the prevention of crosspollination. This is affected in a number of ways. Firstly, the two populations, although sympatric in distribution, may be separated physically by the mere fact that they inhabit different ecological niches according to their needs, and thus are ecologically isolated. Secondly, the flowering periods of the populations living in the same area may not coincide and hence they escape interbreeding. Lastly, the floral mechanisms of certain species of plants render cross-pollination impossible.

Internal isolating mechanisms operate after pollination. In fact, a large number of plant species are cross-pollinated by various agents but it is only rarely that a few of them interbreed.

All the barriers under this category are concerned with many kinds of reactions produced within the plant tissues as a result of the contact of two different genotypes. The first barrier which begins to work just after pollination is one preventing fertilization and the second inhibits the development of the hybrid seeds. Both are together referred to barriers to crossability. There is another group of internal barriers operating within the hybrids themselves. These barriers eliminate the hybrids at various stages of their life cycle. The hybrid seedlings die quite early; or, if they grow, they are very weak and do not reach flowering stage; or they are sterile. And lastly, if by chance the hybrids are able to set seeds, the later progeny are rendered unfit to complete for survival. Isolating mechanisms can be summarised as follows (a slight modification of Stebbins, 1950, p.196):

External Isolating Mechanisms	I. Geographical Isolation
	II. Physiological Isolation
	A. Barriers to pollination
	1. Ecological Isolation
	2. Temporal and Seasonal Isolation
	3. Mechanical Isolation
Internal Isolating Mechanisms	B. Barriers to Crossability
	1. Pre-fertilization barriers
	2. Post-fertilization barriers
	C. Barriers in the hybrids
	1. Hybrid inviability or weakness
	2. Failure of flowering in the hybrids
	3. Hybrid sterility
	4. Inviability and weakness of F2 and later segregates

It has already been noted that the European species of Epilobium are sympatric in distribution and that in some cases their habitats are hard to distinguish. Although it has been suggested that self-pollination in Epilobium is an efficient barrier against interbreeding (Valentine, 1951; Lewis and Moore, 1962), this does not account for the occasional appearances of natural hybrids in the populations. Moreover, there are species in the genus which are exclusively cross-pollinated. Also, the self-pollinated species are probably cross-pollinated to some extend. It is thus obvious that some of the important barriers which operate to maintain Epilobium species are internal ones. One of the main aim of this thesis has been therefore to study the internal isolating mechanisms working in the genus.

Barriers to crossability are basically of two types; (i) prefertilization barriers which inhibit fertilization, and (ii) post-fertilization ones which arrest the development of embryo and endosperm. The prevention of fertilization is accomplished in many ways:

(i) Pollen does not germinate on foreign stigmas.

(ii) Pollen tubes fail to traverse the entire length of the style and consequently fail to reach the ovary.

(iii) Pollen tubes burst or die before they reach the ovary.

(iv) Male gametes do not fuse with the egg cell and the polar nucleus.

The factors working to this end may be summarised as pollen incompatibility. A good review of pollen incompatibility in flowering plants has been given by Lewis (1949). This has a genetical basis and in many plant species, which have been investigated, it has been found to be controlled by a single gene S with several alleles, each having a specific function; but this is mainly associated with outbreeding, or it is not generally important as an interspecific isolating mechanism at least so far as closely similar species are concerned.

In the European species of Epilobium, none of the above phenomena associated with pollen incompatibility has ever been suspected to be a major obstacle to hybridization. There is evidence that pollen germinates easily and in very high percentage after cross-pollination between different species (Lewis and Crowe, 1958). All experiments so far made to synthesize hybrids have shown that pollen incompatibility, if it works at all, has little effect. It is due to this that more attention has been paid to the post-fertilization barriers in the present study. However, there are reasons to believe that effects of pollen incompatibility cannot totally be ignored. Practically nothing is known about the comparative rates of pollen tube growth within styles of different species, although it has been shown that growth rate is frequently slower in an incompatible cross (Sanz, 1945; Avery, Satina, and Rietsema, 1959; Grant, 1963). It is on this basis that an attempt has been made to explain the role of pollen incompatibility causing lower seed set (only partly) in some of the crosses.

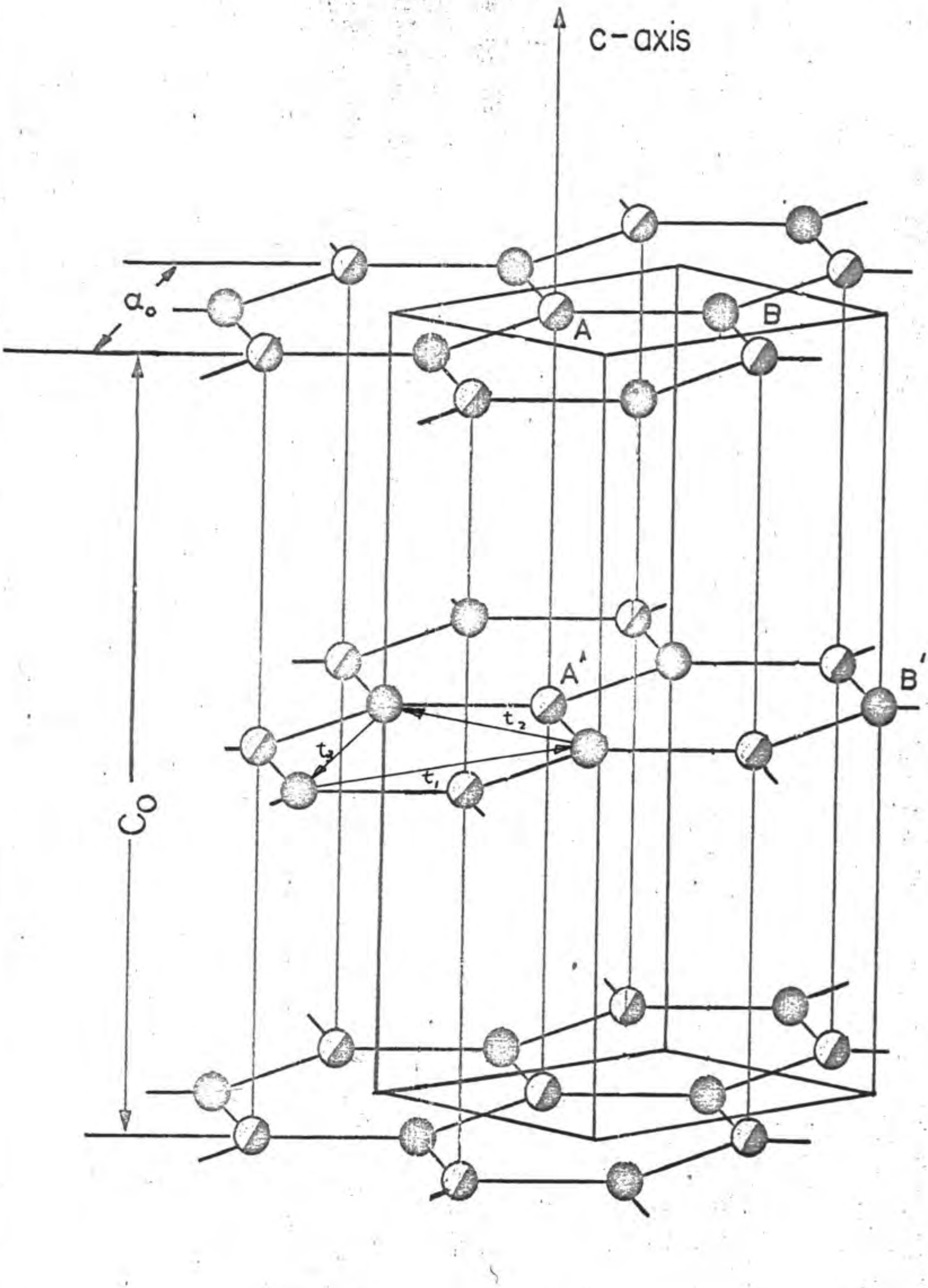
Post-fertilization breakdown, or seed incompatibility as it has been called (Valentine, 1953), has been rather neglected by geneticists and plant breeders. Perhaps the study of the development of the embryo and its related tissues is entirely an embryologist's job!

As early as 1925, Michaelis reported many histological abnormalities in seed development following interspecific crosses in Epilobium. He found some adventive budding of cells within the embryo sacs and hypertrophy of the nucellus in the crosses, E. hirsutum x angustifolium, E.montanum x angustifolium, and E.hirsutum x Dodonaei etc. Besides, degeneration of the embryo was quite common in each case. Such abnormalities have also been recorded in Oenothera (Renner, 1914), in Iris (Sawyer, 1925), in Nicotiana (Christoff, 1928) and in many other plants. Usually the patterns of abnormalities have been different in reciprocal crosses. Nobody explained the causes of all these, nor was it known how these were responsible for the collapse of the seed.

Brink and Cooper (1940) put forward a theory to explain the role of the histological abnormalities observed in Medicago crosses. They maintained that any abnormal growth of tissue other than that essential for normal seed would disturb the balance of food supply to the developing embryo, endosperm and the surrounding maternal tissue (integument). They had noted a marked meristematic activity in selfed M. sativa (which is self-incompatible) in the inner integument which became many-celled thick, instead of only 2-celled thick in normal seeds, absorbing most of the nutrients for itself and starving the embryo and the endosperm. This type of seed failure was termed somatoplastic sterility. So in their opinion, regular and needful supply of nutrition to each of the three developing components of the seed, initiated as a result of fertilization, was vital. Eventually they dwelt upon the great importance of endosperm in seed development.

In a review, Brink and Cooper (1947) concluded that the endosperm was vital to the early stages of seed development. A double contribution (fusion) in the polar fusion nucleus plus another fusion of a third nucleus, i.e., one of the male gametes, during the secondary fertilization, together give the endosperm a physiological advantage over the maternal tissue for nutrient competition. Recently this hypothesis of seed failure due to disturbed nutritional relations between endosperm and maternal tissue has been re-emphasized by Håkansson (1956). Any way, this approach is based primarily on histology and to some extent on the cytology of hybrid embryo and hybrid endosperm. It does not explain why these abnormalities should arise in the first place.

FIGURE I



exact nature of disturbances and their precise function and control are not known. From this point of view, information regarding what happens during seed development in various crosses in *Epilobium* is of interest; and a thorough examination, stage by stage, of the developing seed has been made.

VIABILITY AND FERTILITY OF HYBRIDS.

Interspecific hybrids may die at the seedling stage; they may show malformations; they may have varying degrees of vigour; and they may have all degrees of fertility. A general review of the subject has been given by Stebbins (1950) and need not be repeated here. We may, however, mention a few points at this stage about interspecific hybrids in *Epilobium*. Apart from the intersectional crosses between *Chamaenerion* and *Lysimachion* which Michaelis (1925) reported as having failed due to seed abortion, almost all combinations of interspecific hybrids have been synthesized in Europe (Compton, 1910, 1911, 1913a, 1913b; Lehmann, 1918, 1919, 1925; Geith, 1924; Mechaelis, 1925, 1931, 1938, 1954; and others). This apparently implies great genetical similarities between all the species. However, the hybrids are often weak and sterile; and the explanation put forward for this situation of hybrid weakness and sterility in *Epilobium* hybrids is based on the special role that the cytoplasm is supposed to play in the genus (Michaelis, 1931, 1954). It is not the disharmonious combinations or direct interactions of the parental genotypes, but rather the interactions between the hybrid genotypes and the maternal cytoplasm which seem to be important.

That the reciprocal hybrids differ in their vigour and fertility has been observed by all who raised artificial hybrids in *Epilobium* and also is not very uncommon in other genera. But how far the cytoplasm is important in the case of *Epilobium* and not so in others is the real problem. Michaelis pollinated the hybrid *E.hirsutum x luteum* as well as the reciprocal with pollen from *E.hirsutum* for several successive generations. By adding successively only *hirsutum* genome to *hirsutum* cytoplasm on the one hand and to *luteum* cytoplasm (in the case of reciprocal) on the other, it was expected that both the lines would converge to perfect similarity. But they did not; *luteum* cytoplasm had still its effects on the other line of hybrids. This shows that cytoplasm is genetically not ineffective and its plasmon cannot be rebuilt by a foreign genome. But it is not only in cytoplasm that species of *Epilobium* differ from each other; there are also differences between them in the nuclear genes. Both Lehmann and Michaelis demonstrated that different strains of *E.hirsutum* as well as those of *E.montanum* and *E. roseum* reacted differently to *E.luteum* and other species. These differences have been shown to be genically controlled (Lehmann, 1939); thus an F_1 hybrid btween two strains of *E. hirsutum*, when outcrossed to

E. adenocaulon, gives two types of interspecific hybrids in the ratio 1 : 1.

The role of cytoplasm in evolution in general has been discussed by Caspari (1948). This particular aspect is of special interest with regard to evolution in Epilobium. Experiments have shown that even interstrain crosses of the same species may result in inviable hybrids. Where are the differences being built up, in cytoplasm or in genome? Is the cytoplasm really independent of the genome? If not, to what extent are the two interdependent? These problems complicate the process of speciation in the genus. The present study does not claim to find final answers to all these problems. Rather an attempt has been made to sort out different stages in the life cycle of the hybrids at which the internal isolating mechanisms are operating. It also seeks to find out how effective these mechanisms are.

A study such as this involves extensive hybridization work. The hybrids obtained have been observed carefully for their morphological characters, compared with their parents, and a correlation between morphological and genetical similarities of the species has been made. The genetical affinities have been measured in terms of crossability (on Valentine's crossability series pattern in Primula) and interfertility of the hybrids.

How far interfertility is a measure of genetic relationship is highly controversial. We have already noted differences in the two kinds of genes or groups of genes operating on two different levels of isolation: one solely concerned with physiological disturbances and the other with reproductive activities. In many cases, failing hybrid embryos have been grown in culture media, the hybrids have grown to maturity and have shown fertility indicating there are no other gene differences except those causing seed failure. Thus the degree of crossability cannot be taken as an absolute measure of genetic relationship, nor can extent of hybrid fertility. On the other hand, these criteria cannot be entirely neglected in a study of relationship between species. They are clearly important from the evolutionary point of view; and they may have at least limited importance from the taxonomic point of view. We shall attempt, later in this thesis, to compare degree of relationship between Epilobium species using these different kinds of criteria. We shall, incidently, also consider briefly another problem, that of the relationship between certain European and New Zealand species. This is a problem of considerable interest from the point of view of knowing the extent to which geographically isolated species can diverge morphologically without at the same time developing sterility barriers.

CHAPTER II

PLANT MATERIAL

ORIGINS OF THE PLANTS USED

E. angustifolium L.-

(i) P16762: Loc. Eastham, Co. Cheshire, England; coll. P.L. Pearson.

(ii) 610: Loc. Kungläv (alt. 80m), Bohuslän prov., Sweden; coll. H. Anderson; seeds obtained through the Botanical Garden, Lund, Sweden.

(iii) 60.31 Force Burn: Loc. Force Burn near the Moor House Nature Reserve, Co. Westmorland, England; coll. M.J. Harvey.

(iv) Durham: Loc. Little High Wood, Durham, Co. Durham, England; coll. V. Thakur.

E. fleischeri Hochst.

(i) U62: Of garden origin, seeds obtained through the University Botanical Garden, Uppsala, Sweden.

E. luteum Pursh.

(i) U261: Of garden origin, seed obtained through the University Botanical Garden, Uppsala, Sweden.

(ii) Birmingham: Of garden origin, obtained through M.J. Harvey.

E. hirsutum L.-

(i) KR5862: Seedlings raised from seeds of a herbarium specimen in the herbarium of the University of Durham, coll. A. Dunk, P. Halliday and A. Uchlin, in 1958 (seeds germinated in

1962) from Towpath between Kew and Richmond, Surrey, England.

(ii) 672: Locality unknown, probably of garden origin, seeds obtained through the Botanical Garden, Karl-Marx University, Leipzig, E. Germany.

(iii) NCl: Locality unknown, seeds obtained through the Department of Botany, the University of Newcastle upon Tyne, England.

(iv) Durham: On the bank of River Wear, near the University Race-course, Durham, England.

E.parviflorum Schreb.

(i) SP6262: Loc. St. Bride's, Pembrokeshire, Wales; coll. D.H. Valentine. The original specimen is deposited in the herbarium of the University of Durham.

(ii) 620: Loc. V. Vram, Tollarp (alt. 45 m), Skåne, Sweden; coll. Ö. Nilsson; seeds obtained through the Botanical Garden, Lund, Sweden.

(iii) 675: Locality unknown, seeds obtained from the Botanical Garden, Karl-Marx University, Leipzig, E. Germany.

(iv) 163: Loc. along the north side of Glencar Lough, Co. Leitrim, Ireland; coll. H.A.P. Ingram.

E.montanum L. -

(i) PL63: Loc. Eastham, Co. Cheshire, England; coll.P.

L. Pearson.

(ii) SL621: Loc. the Science Labs. garden, Durham, England; coll. D.H. Valentine.

(iii) 616: Loc. Landskrona, Erikstrop (alt. 4 m.), Skåne, Sweden; coll. A. Nilsson; seeds obtained through the Botanical Garden, Lund, Sweden.

(iv) 617: Loc. Tylöskogen (alt. 225 m.), Godegård, Östergötland Sweden; coll. A. Nilsson; seeds obtained from the Botanical Garden, Lund, Sweden.

E. lanceolatum Seb. & Mauri.

(i) AFW604: Of garden origin, young plants obtained through Miss A.F. Wood, Bourne End, Bucks, England.

E. roseum Shreb.

(i) Skåne 621: Loc. Landskrona (alt. 5 m.), Skåne, Sweden; coll. A. Nilsson; seeds obtained from the Botanical Garden, Lund, Sweden.

E. adenocaulon Hausskn.

(i) SL: Locally growing in the Science Labs. garden, Durham, England.

E. adnatum Griseb.

(i) Pn661: Loc. in between Tickenham and Nailsea, Somerset, England; coll. T.D. Pennington.

(ii) 611: Loc. Gorslöv, Nordana (alt. 20 m.), in Skåne, Sweden; coll. J. Johansson; seeds obtained through the Botanical Garden, Lund, Sweden.

E. tetragonum L.

(i) PS163: Loc. Sacavèm, Portugal.

E. Lamyi F. Schultz.

(i) 15: Loc. outskirts of Wells, Somerset, England; coll. T.D. Pennington.

E. obscurum Schreb.

(i) Pn763: Loc. Langbottom Farm, Shippan, Somerset, England; coll. T.D. Pennington.

(ii) Durham: Locally growing in the Science Labs. garden, Durham, England.

E. palustre L.

(i) 618: Loc. Fågeltofta, Kronovall (alt. 100 m.), in Skåne, Sweden; coll. Ö. Nilsson; seeds obtained through the Botanical Garden, Lund, Sweden.

(ii) 619: Loc. Görstöv, Nordanå (alt. 18 m.), in Skåne, Sweden; coll. J. Johansson; seeds obtained through the Botanical Garden, Lund, Sweden.

E. anagallidifolium Lam.

(i) R1621 a. Loc. Moorhouse Nature Reserve, Westmorland, England; coll. P.H. Raven; growing in the Science Labs. garden.

E. alsinifolium Vill.

(i) R1621 b.: Loc. Moorhouse Nature Reserve. Westmorland, England; coll. P.H. Raven; growing in the Science Labs. garden.

E.nertericides, Cunn.

(i) Scotland: Of garden origin, Edinburgh, Scotland; coll. young plants by Miss E.P. Beattie.

E. brevipes f. Hook.

(i) Birmingham: Of garden origin, young plants sent by M.J. Harvey.

Cultivation:

As is evident from the above list, all the plants (except four) were grown from seeds collected in the wild or obtained from various Botanical gardens. The seeds were put on moist filter paper in petri-dishes for germination. When the seedlings were about 10-15 days old, they were transferred to pots. The pots were kept in a warm greenhouse and watered regularly. This method succeeded in producing the maximum number of healthy and strong seedlings from a small supply of seeds. If the seeds are directly sown in the soil, the germination is slightly lowered and if, by chance, they happen to be buried deep in the soil, they hardly germinate.

The seedlings were transplanted into boxes. Those meant for breeding were all grown in an insect-proof greenhouse, while others were grown outside.

CHAPTER III

POLLINATION MECHANISM AND BREEDING SYSTEM

Breeding Systems in the Species used in the Present Study:-

Techniques of breeding to be employed in hybridization experiments depend much on the breeding systems of the species concerned. Emasculation may not be necessary if self-pollnation is not effective. If necessary, when and how to emasculate depends on the time of maturation and dehiscence of anthers and the position of the stamens in the flower, inflicting minimum or no injury to the flower itself and to the pistil in particular. Thus a sound knowledge of floral structure and pollination mechanism in the species used in the present work was most desirable.

Brockie (1959) has published an excellent account of the breeding systems in the New Zealand Epilobia, all of which, according to him, are chiefly self-pollinated. However, it has to be borne in mind that the New Zealand Epilobia belong exclusively to Synstigma and that the same story may not hold true for members, belonging to Schizostigma and the Sect. Chamaenerion, which have 4-lobed stigmas. In the literature, E. angustifolium is described as undoubtedly a cross-pollinated species. So is E. hirsutum, but forms that are usually self-pollinated are also present in this species. A detailed description of pollination in these two and a few other European species of Epilobium is to be found in Knuth's Handbook of Flower Pollination (1908, Vol. II, pp. 441-447). This book mentions (and so does Mr. Brockie) the fact that weather fluctuations and altitude have spectacular effects on ripening of anther and stigma and on flower opening, thereby altering conditions of pollination and its mechanism. This is well supported by our observation at Durham of the plants used in the present work.

Floral Structure.

The flower is tetramerous; zygomorphic in E.angustifolium, subzygomorphic or nearly actinomorphic in E.fleischeri, and actinomorphic in others. In Table 2 are recorded, besides other information, average diameter of the flower and its colour for each species. It will be noted that the flowers vary a great deal in size from about 3 cm. in diameter in E.angustifolium or E.fleischeri to 4 mm. in E.nerterioides. Large size and bright colour could be important factors for insect visits.

There are 8 stamens in two rows. The four antesepalous stamens are longer and mature earlier than the four antepetalous ones.

The stigma is either entire or 4-lobed. Different kinds and structures of the stigmas found in the plants under study are illustrated in Fig. 1. The four long stamens are usually at level with the stigma, but in some cases they are shorter or very rarely they project beyond it. This relative position of the longer stamens and the stigma as observed in freshly open flowers has been given in Table 2 for each species. Within 24 hours after the flower has opened, the stamens acquire considerable length and the shorter ones too get within the reach of the stigma next day.

Pollination:

Self-pollination is dependent on two factors: (i) the stigma and the anther must come into contact with each other, and (ii) both must be ripe together or at least they must overlap in their maturation period. In absence of either condition, self-pollination will not materialize. Instead cross-pollination will occur should insect visitation take place. What has been observed to be the usual mode of pollination in all our plants and the timing of maturation of anther and/or stigma in them has been shown again in Table 2.

It is evident from the table that the species listed therein fall distinctly into 3 groups; *viz*. (i) those which are strictly cross-pollinated and under no circumstances resort to self-pollination, (ii) those which are likewise strictly self-pollinated, and (iii) those which are either chiefly cross-

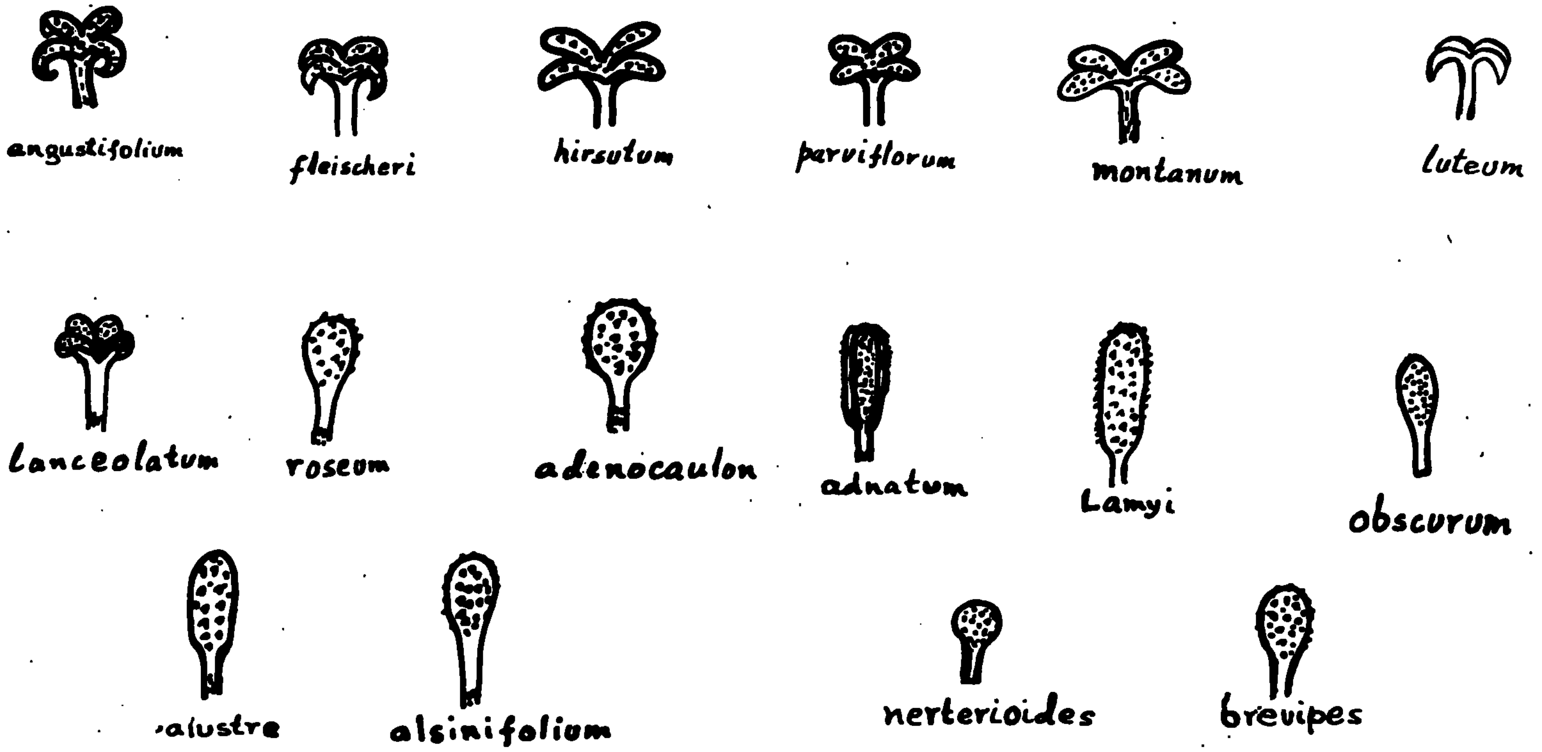


Fig. 1. Stigmatic structure in Epilobium species. All x6.

pollinated or self-pollinated but change over from one mechanism to the other as the circumstances permit.

(i) Strictly Cross-pollinated. Plants of E.angustifolium and luteum, when grown in an insect-proof greenhouse, failed to set seed. What happened was that the anther and the stigma never got into contact. Their position in the flowers of the two species has been illustrated in Fig. 2 a-e.

In E.angustifolium, it is the peculiar movement of the stamens and the style occupying different positions in the flower at different times that precludes meeting of the anther and the stigma. In young flowers the style is short and bent downward (Fig. 2a). Gradually it straightens, lengthens, and spreads out its stigmatic lobes. In older flowers it appears to be longer than the stamens which by this time have already discharged their pollen and are themselves curved downward (Fig. 2b, c). When the insect visits the young flower, it sits on the stamens which provide convenient alighting place to approach through to the nectaries situated at the base of the style flanked by the filaments. In doing so, the insect gets loaded with pollen. When the flower is old and the stamens are curved downward and shrivelling, the now straightened style provides the alighting place to the insect visitors which carry loads of pollen with them. Thus cross-pollination is affected.

TABLE 2

Important floral characters and Pollination in Epilobium species

	Species	Flower colour	Flower diameter in mm.	Stigma	Length of stamen in relation to stigma	Maturation of Anther	Pollination
1	E. angustifolium P16762 60.31	Purple-red	20-30	4-lobed	Stigma exceeding stamens	Protandrous	Cross-pollinated visited by insects.
2	E. fleischeri U62	Purple	35-40	"	"	"	"
3	E. hirsutum KR5862 672 NC1	Dark Purple	15-23	"	Stigma exceeding the longest stamens	Protandrous NC1 - homogamous	Mainly cross-pollinated visited by flies
4	E. parviflorum SP6262 675 620 163	Pink	6-9	"	Stamens at level with stigma	Homogamous	Commonly self-pollinated (visited by hive-bees and occasionally cross-pollinated)
5	E. montanum SL621 B16201 PL63 616	Pink	6-9	"	" (or stamens longer)	"	Commonly self-pollinated (cross-pollinated occasionally)
6	E. lanceolatum AFW604	Whitish pink	6-7	"	Stamens at level with stigma	"	Commonly self-pollinated
7	E. luteum U261	Yellow	18-25	"	Stigma exceeding stamens	Protandrous	Cross-pollinated
8	E. roseum 621 Skåne	Rose colour	4-6	Entire	Stamens at level with stigma	"	Self-pollinated
9	E. adnatum Pn661 E. tetragonum PS163	Pink Pink	6-8 8-10	"	Stamens longer	Protandrous Homogamous	Self-pollinated
10	E. Lamyi 15	Pink	10-12	"	"	Protandrous ± Homogamous	Self-pollinated
11	E. obscurum Pn763	Pink	7-9	"	Stamens at level with stigma	Homogamous	Self-pollinated
12	E. adenocaulon SL	Pink	4-6	"	"	Homogamous	Self-pollinated
13	E. palustre 618, 619	Pink	4-6	"	"	Homogamous	Self-pollinated
14	E. alsinifolium R1621	Pink	8-9	"	"	Homogamous	Self-pollinated
15	E. nerterioides Scot.	White	3-4	"	"	Homogamous	Self-pollinated
16	E. brevipes Birmingham	Purple	10-12	"	"	Homogamous	Self-pollinated

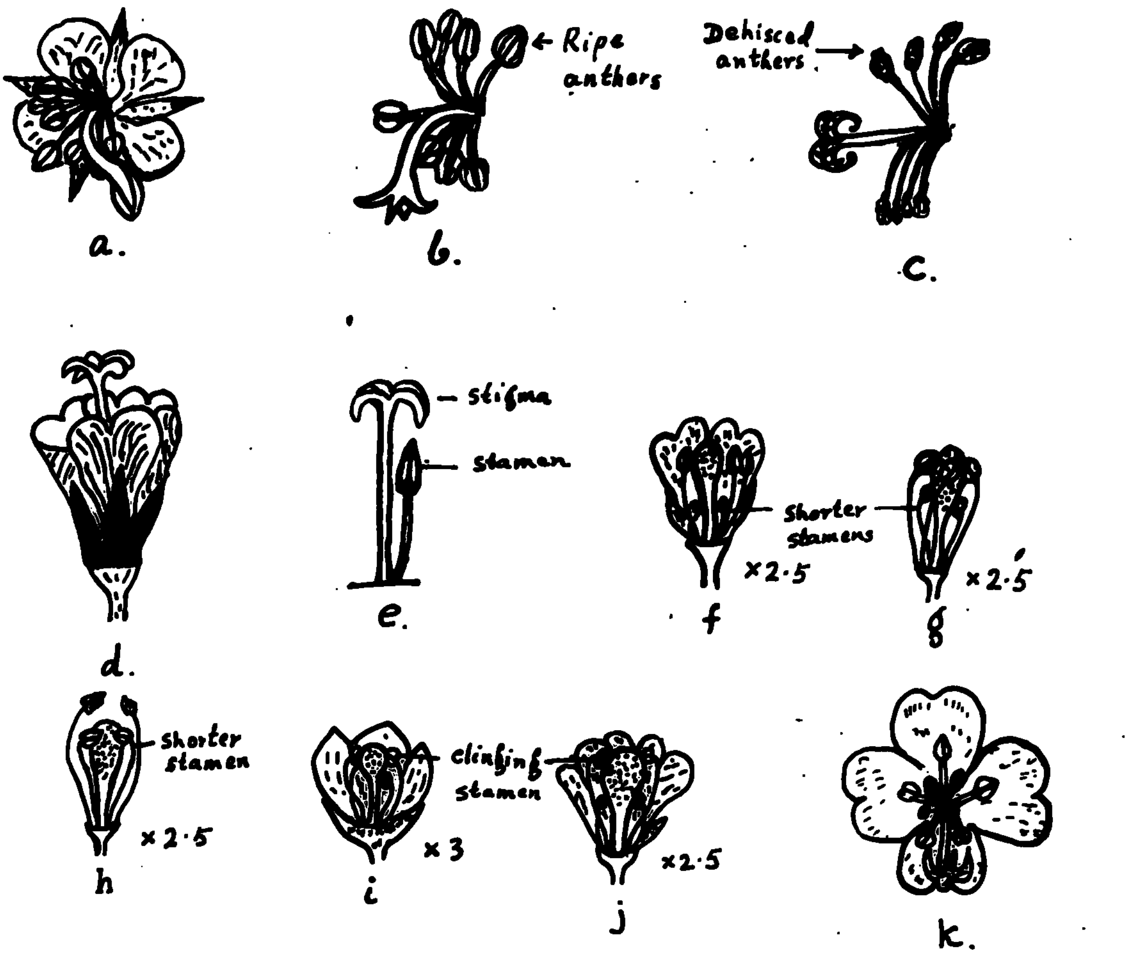


Fig. 2. Diagram showing position of stamens vis-a-vis stigma. a-c *E. angustifolium*, c dehisced anthers; d,e *E. luteum*, e stamen shorter than stigma; f-h *E. obscurum*, f, on the first day of opening of flower, g at night, h on the next day; i *E. nerterioides*; j *E. roseum*; k *E. hirsutum*. b, c, e, g and h with no petals.

In E.luteum, the style is much longer than the stamens and it remains so throughout (Fig. 2 d, e). The stigma projects beyond the flower. The flower is more or less tubular and to reach the nectaries the proboscis of the insect while sitting on the style must pass through the dehiscing anthers. When the insect moves on to the next flower, it alights again on the stigma and transfers the foreign pollen carried over from the previous flower.

(ii) Strictly Self-pollinated. Species listed from 8 to 16 in Table 2 are decidedly self-pollinated. Plants kept in the insect-proof greenhouse yielded almost equal amount of seeds per capsule as those grown in the open. The mechanism of opening and closing of the flower in each of the species observed leaves no chance for cross-pollination. If it occurs, it is likely to be ineffective, because selfing will already have taken place.

The flowers are most commonly homogamous. On a hot sunny day, a definite trend of protandry is observed in the flowers of E.roseum, adnatum, Lamyi and adenocaulon. Dull weather delays ripening of the anthers. Thus one has to be very careful in choosing buds of the right size for emasculation in different weather conditions. The bud of the size found suitable for emasculation in dull weather condition usually contains dehisced anthers if it is bright and sunny. Brockie (1959), who found all the New Zealand Epilobia (which are self-pollinated) to be protogynous, also noticed this inconsistency in relative maturation of the anther and the stigma. To me it appears to be of little significance whether the anther or the stigma ripens first. What is most vital in these species is the duration of the receptivity of the stigma and availability of pollen in the flower during that period.

It has been mentioned earlier that the two rows of the stamens often mature one after the other. By the time the shorter stamens are ripe, the stigma is also by all means receptive. Thus there is a good overlap of time in maturation of the two. Secondly, all the stamens do not dehisce simultaneously, they continue to discharge pollen over a long period of time. Likewise the stigma also remains receptive for a long period. I have pollinated stigmas at different times after emasculation and obtained seeds in each case. Under these circumstances, what remains to be done is to bring the anther and the stigma toghether. This is achieved by opening and closing of the flower itself.

The flowers open at about 8 a.m. on a sunny day and close again by 4 or 5 p.m. They remain closed during the night and re-open next morning. With the approach of the second evening, they close for ever and fall off. The effect of this opening and closing of the flowers in E. obscurum on mutual contact of the anther and the stigma has been illustrated in Fig. 2 f-h. On the first day when the flower opens, the longer stamens are mature and at the same level with stigma (Fig. 2f.). They begin to dehisce with the progress of the day and when the flower closes all the pollen is deposited on the stigma (Fig. 2 g). The shorter stamens, by this time, have reached the stigma and depending on temperature they dehisce before re-opening of the flower next morning or cling to the stigma and gradually discharge pollen on it (Fig. 2h). In hot weather condition, the anthers dehisce in the bud and self-pollination takes place much before the flower opens.

When there is prolonged bad weather, the flowers of E. adnatum, Lamyi, tetragonum, palustre, brevipes and nerterioides hardly open and self-pollination is inevitable as a result of this pseudo-cleistogamy.

Anthers of E. nerterioides (Fig. 2 i) and E. roseum (Fig. 2 j) always cling to the stigma, discharge pollen on it and thus self-pollination is effected. In Knuth's Handbook (l.c. pp. 446-67) it is mentioned that the ripe anthers and stigmas in some flowers of E. roseum are far apart and hence insects can effect cross- and self-pollination with equal facility. The possibility of cross-pollination is also mentioned therein

(p. 446) in E.alsinifolium if the slightly protogynous flowers are visited early by insects. I found the anthers were very close to the stigma in E.alsinifolium and the flowers were perfectly adapted for automatic self-pollination.

(iii) Partly Cross-pollinated and Partly Self-pollinated. We shall notice that there are two kinds of species involved in this category. E. fleischeri and E. hirsutum are chiefly cross-pollinated and self-pollination occurs only occasionally in them. On the other hand, E. parviflorum, montanum, and lanceolatum are commonly self-pollinated but also susceptible to occasional cross-pollination.

The floral mechanism of E. fleischeri is essentially the same as in E. angustifolium, but the diverging lobes of the stigma, somehow or other, touch the dehiscing anthers in the greenhouse. In the open, insects effect cross-pollination much before the anthers and the stigmatic lobes get into contact. In E. hirsutum (Fig. 2 k), the stigma is higher than the long stamens and therefore only corss-pollination can take place. However, all the samples grown in the insect-proof greenhouse were able to set a fair amount of seed and it was observed that the anthers and the stigma couldmake contact. In fact, one form, NCl, has a stigma more or less equalling the longer stamens. Information is available about forms in which the stigma hardly exceeds the longer stamens. The relative length of the two varies from locality to locality.

The flowers of E. parviflorum and E. montanum are homogamous and the longer stamens are at the same level with the stigma. Normally self-pollination occurs, but a number of insects visit their flowers in the open, alight on the conspicuously 4-lobed stigma and effect cross-pollination as well. Not many insect visitors were seen in E.lanceolatum, but most probably it is also open to cross-pollination.

Conclusions

We find that the different species of Epilobium have different pollination mechanisms. E. angustifolium, fleischeri, luteum, and hirsutum, which are mainly corss-pollinated, have large and conspicuously coloured flowers. The most significant point to be noted is that the species that are wholly or partly cross-pollinated have 4-lobed stigmas; whereas the species with entire stigmas are mainly self-pollinated. Further implications of this will be discussed later.

CHAPTER IV.

INTERSPECIFIC HYBRIDS

A. Natural Hybrids.

The occurrence of hybridization between different species of *Epilobium* had long been suggested by the existence of morphologically intermediate plants in natural populations. Hooker in his *Handbook of the New Zealand Flora* (1864, p.76) wrote: "The species are, without exception of locality or country, extremely variable, and probably hybridize." Focke (1881), Haussknecht (1884), and Rubner and Beger (1925) have recorded a large number of spontaneous hybrids involving many species. These are, no doubt, primarily European records, but they do suggest an enormous range of crossability that possibly exists between the species.

Of hybrids outside Europe, we have but little information. Haussknecht (*l.c.*) has mentioned only 9 of them, 3 each from America, Asia and Oceania, including 2 doubtful ones from Asia. Cockayne and Allan (1934) reported 13 hybrid groups from New Zealand, but Allan (1961, p.281) has expressed doubt over the validity of these hybrids. Considering the fact that there are about 50 species of *Epilobium* in New Zealand, nearly more than twice as many as in Eureop, it is surprising that hybrids are so rare. Brockie (1959) points out that the New Zealand Epilobia are self-pollinated. This may account for the apparent rarity of hybrids in New Zealand. Recently Lewis and Moore (1962) described a case of natural hybridization between E.adenocaulon and E.brevistylum at one locality in the Rocky Mountains, North America. The two species frequently occur together in many localities, but hybrids are rarely met with. They are self-pollinated no doubt, but an occasional cross-pollination cannot altogether be prevented. According to Lewis and Moore, the most reasonable explanation of this rarity is the inability of the interspecific hybrids to establish themselves in the habitats normaly frequented by the parents. Rarity of hybridization among Epilobium species in Turkey is explained by Davis (1962) as due to their more restricted distribution, particularly with regard to habitat. They have less opportunity to hybridize and are subject to more rigorous competition.

Frequency of Hybrids in Europe:

Some reports indicate that the frequency of hybrids in Europe varies from locality to locality. Haussknecht (l.c., p.63) says that E. hirsutum hybridizes more easily in North than in South Germany. According to him (l.c., p.26) E.obscurum x palustre in some localities is so vigorous that the parents are suppressed; or on some soils E.obscurum remains but E.palustre disappears. On the other hand, Rubner and Beger (1925) have found this hybrid to be rare. Likewise the frequency of different combinations of hybrids is different in nature; some are formed more easily than others. Rubner and Beger (l.c.) note that hybrids between E.hirsutum and Synstigma are rare, while those between E.hirsutum and Schizostigma appear quite frequent. It is also noticeable that most of the hybrids involving E.hirsutum are among the rarest. Table 3 shows an arrangement of some of the hybrids in order of their frequency of occurrence, compiled on the basis of data taken from Focke and Rubner and Beger. These hybrids involve six species very common also in Great Britain.

Hybrids in the British Isles:

According to Clapham, Tutin and Warburg (1962), almost all combinations of Epilobium hybrids occur in the British Isles. Dandy (1958, pp. 64-66) has given a list of all the hybrids recorded so far in this country. A record is also maintained in the British Museum (Natural History), but it appears that many of the hybrids, although represented in the BM herbarium, have not been included in the chart; it is given here in a revised form as Table 4.

The present image of the genus and its hybrids in Great Britain owes much to the Rev. E.S. Marshall. Almost contemporary to Haussknecht, he developed a keen interest in the British Epilobia. He collected specimens of the species and their hybrids

TABLE 3

Frequency of Natural Hybrids

Rare (Focke and Rubner & Beger agree) or not found.	Rare (Rubner & Beger); Occasional (Focke).	Frequent	Most frequent
hirsutum x adnatum hirsutum x roseum hirsutum x montanum hirsutum x palustre (not recorded by Rubner & Beger) hirsutum x obscurum (no record at all) adnatum x obscurum montanum x palustre	hirsutum x parviflorum hirsutum x palustre	parviflorum x adnatum parviflorum x obscurum parviflorum x palustre adnatum x roseum adnatum x palustre obscurum x roseum obscurum x montanum	parviflorum x roseum parviflorum x montanum obscurum x palustre roseum x montanum

TABLE 4

1		1											
2		+	2										
3		+	+	3									
4		+	+	+	4								
5			+	+	+	5							
6		+	+	+	+		6						
7		+	+	+	+		+	7					
8		+	+	+	+	+	+	+	8				
9		+	+	+	+				+	9			
10		+	+			+			+		10		
11				+	+				+	+		11	
12									+	+		+	12

Herbarium records of hybrids in the British Museum.

\+ Denotes presence of hybrids.

1. E. hirsutum	7. E. Lamyi
2. E. parviflorum	8. E. obscurum
3. E. montanum	9. E. palustre
4. E. lanceolatum	10. E. adenocaulon
5. E. roseum	11. E. alsinifolium
6. E. adnatum	12. E. anagallidifolium

from all over the country and was able to show that nearly all the British species of the genus hybridized in nature. His vast collections preserved in the herbaria of the British Museum, Kew, and the Botany School, Cambridge, bear witness to his excellent work.

An examination of the available herbarium material, mainly at the British Museum and Cambridge, gives some valuable information:

1. Most of the specimens are from rather few localities, so that no estimate of hybrid frequency can be made. However, the following hybrids appear to be well represented in the herbaria from several localities and therefore may well be regarded as relatively more frequent than others:

E.parviflorum x montanum
E.parviflorum x roseum
E.montanum x obscurum
E.montanum x roseum
E.obscurum x palustre

This exactly conforms to the European pattern (see Table 3).

2. Diagnosis of hybrids in the herbaria seems to have been made on basis of intermediate morphology and apparent sterility. This has allowed many errors to creep in and consequently identity of many of the hybrids is open to doubt. The late

Mr. G.M. Ash has frequently expressed disagreement over Marshall's identification. Judging from the shape of the stigma and my experience with the artificial hybrids, in many cases, I agreed with Ash.

(a) Of the hybrids in the herbaria, the following seemed to me to be very dubious or not hybrids:-

i. E.hirsutum x lanceolatum - This is recorded as a 'new hybrid' (see below) by E.S. Marshall. The specimen at the British Museum appears to have the benefit of doubt, but the one (and there is only one) at Cambridge has imperfectly 4-lobed stigma and is decidedly not a hybrid of this combination.

ii. E.montanum x palustre - The specimen at the British Museum is most likely E.palustre. The stigma is entire, rather than obscurely 4-cleft.

iii. E.lanceolatum x adnatum - According to Mr. Ash, the BM specimen is E.adnatum. The two at Cambridge are also doubtful.

iv. E.lanceolatum x alsinifolium - All available specimens of this hybrid are highly doubtful.

v. E.roseum x adenocaulon - Only one specimen at BM; highly doubtful.

(b) Of the hybrids in the herbaria, a considerable confusion prevails in the following hybrid groups:-

E. parviflorum x adnatum
E. parviflorum x Lamyi
E. lanceolatum x Lamyi
E. Lamyi x obscurum

The confusion results due to a lack of sharp distinction between hybrids of *E.adnatum* and those of *E.Lamyi*. Many specimens of *E.parviflorum x obscurum* and *E.parviflorum x roseum* have also been confused with *E.parviflorum x adnatum* and *E.parviflorum x Lamyi* perhaps as a result of complex hybridization.

3. There is strong evidence that complex hybrids are represented in the herbaria. The following triple hybrids have rightly been identified by E.S. Marshall:

E.(obscurum x parviflorum) x Lamyi - a single specimen, No. 105, lying with *E.parviflorum x obscurum* hybrids at Cambridge.

E.(montanum x roseum) x parviflorum - a specimen lying with *E.montanum x roseum* hybrids at Cambridge.

Besides, *E.(montanum x obscurum) x parviflorum* is undoubtedly present among *E.parviflorum x obscurum* hybrids in the herbaria.

4. In addition, there are certain groups of specimens, representing populations from a single locality, which can be

reasonably interpreted as a hybrid swarm or including backcross segregates. These are:-

E.montanum x parviflorum
E.montanum x roseum
E.montanum x obscurum
E.parviflorum x roseum
E.parviflorum x obscurum
E.obscurum x palustre
E.alsinifolium x palustre
E.alsinifolium x anagallidifolium

5. The following hybrids, found only in Britain, have so far been not reported from elsewhere:

E.hirsutum x lanceolatum (see dubious hybrids)
E.hirsutum x palustre (= x E. Waterfalli)
E.obscurum x anagallidifolium (= x E. Marshallianum)

Chamaenerion Hybrids:

No hybrids between species of Chamaenerion and those of Lysimachion are known to occur in nature. Hybrids between species of Chamaenerion themselves were also not known until Böcher (1962) reported a hybrid population of E.angustifolium x latifolium in West Greenland. Long before, Jeffrey (1916) and Holden (1916) had described pollen sterility in natural populations of E.angustifolium in South of England, but they ascribed this to intervarietal crosses within the species occurring in nature rather than to interspecific hybridization.

CHAPTER IV

B. ARTIFICIAL HYBRIDS

Survey of previous work:-

Perhaps the first recorded attempt to raise an artificial hybrid of Epilobium, as one gathers from Compton (1911), was made as early as 1842 by Bell Salter. An old belief that E.roseum possibly came out of "seeds of E. tetragonum impregnated by the pollen of E.montanum" was shattered when Bell Salter found the hybrid resulting from the above cross was not identical with E.roseum! Focke (1881, p. 528) mentions two hybrids, E. montanum x roseum and E. montanum x obscurum, which were made by him. Haussknecht (1884, p. 27) also reports that he raised two hybrids: E.Lamyi x montanum and E.montanum x parviflorum, each made in both directions. However, it was Compton (1910, 1911, 1913a, 1913b), who gave more authentic and elaborate details of the experimental hybrids for the first time. It must be emphasized here that Compton began his experiments at a time when the Mendelism had just been revived and his approach was less biased taxonomically than that of Focke or Haussknecht. Very critical of the process of identification of a supposed hybrid on assumed intermediateness of characters, he (1911) warned: "Recent experimental work has demonstrated that a cross-bred is by no means intermediate between its parents; it may even be indistinguishable from one of them, and show no trace of the other. The phenomena of dominance and the Mendelian segregation of unit characters are of greatest importance to students of naturally occurring hybrids." The crosses made by Compton are summarized below in Table 5.

Compton's important results are that the hybrids are highly sterile and that there are sometimes reciprocal differences. He very carefully noted all the characters of the hybrids and considered their mode of inheritance. Long runners of E. hirsutum are always suppressed whenever this species is crossed in either direction with E.montanum or even only as female with E. adnatum, both of which have no runners. While in the hybrids of hirsutum and montanum long simple hairs of hirsutum appear to be recessive, the hirsutum x adnatum hybrids possess neither the long erect hairs of hirsutum nor the closely appressed down hairs of adnatum but a third type, intermediate between those of the parents, obliquely directed upwards. An obscurely 4-cleft stigma comes out of a union between Schizostigma and Synstigma, but the degree of notching varies according to the species used.

Hybrids between E. hirsutum and montanum are particularly very interesting. Compton (1913a) observed that they were reciprocally different in many respects when they were first produced, but the differences narrowed down in course of time. The hybrid E. hirsutum x montanum was dwarf, inhibited and

TABLE 5

Year	Hybrid-combination (The seed parent first)	Pollen fertility	Seed set	Special features
1910	hirsutum x adnatum f. stenophylla	nil	nil	Differs from Haussknecht's details of the natural hybrids
1911	adnatum x montanum	-	nil	Resembles Haussknecht's description of the natural hybrids.
1913a	hirsutum x montanum	No perfect flower ever produced		Variable offspring.
	montanum x parviflorum	good pollen	a few seeds	Perfectly intermediate between the parents.
	hirsutum x parviflorum	a few good pollen	"	"
1913b	montanum x hirsutum	"	nil	Differs from the reciprocal hybrid.

unable to flower, and could survive only in cultivation by means of vegetative propagation. The reciprocal, on the other hand, was vegetatively vigorous and produced normal flowers. At the end of the third year, some shoots resembling the reciprocal hybrid came out of the two stocks of E. hirsutum x montanum. This is why Compton does not seem to have attached much significance to the reciprocal differences.

However, later workers found that differences between the reciprocal hybrids of Epilobium were very significant. Lehmann (1918) obtained two types of hybrids from reciprocal crosses of E. parviflorum and E. roseum. The cross parviflorum x roseum yielded a hybrid, which he called E. rigidum completely sterile on both male and female sides. The reciprocal E. curvatum (= roseum x parviflorum), on the other hand, was capable of setting about 35-40 seeds per capsule (cf. c. 190-200 in E. parviflorum and 150 in E. roseum) and also produced a large amount of good pollen. Almost the same pattern of results followed in other crosses made by Lehmann (1919). For example, two distinct types of hybrids were again found in crosses between E. parviflorum and montanum. The hybrid E. parviflorum x montanum (= E. triste) produced almost no seed at all; whereas about 50% well-formed seeds were contained in the capsules of E. montanum x parviflorum (= E. suave). The reciprocals of E.parviflorum-palustre- crosses were not very different in gross morphology but differed in sterility; the E.palustre x parviflorum hybrid had a fair amount of good pollen and set a fair proportion of good seeds in contrast to the reciprocal which was both male and female sterile. Thus whenever parviflorum was mother, observed Lehmann (1919), the hybrid tended to be practically sterile, but this did not happen when it was used as a father. Also the hybrids with parviflorum as their mother had petals usually reduced in size. Many more hybrids of several combinations exhibiting more or less the same pattern of reciprocal differences in their reproductive capacity were obtained by Renner and Kupper (1921), Geith (1924), Schwemmle (1924), and Oberreuter (1925).

Frequent appearance of lethal and crippled hybrids in Epilobium crosses has become another important feature of interest. Very often the reciprocal differences are manifest also in kinds and degrees of inhibitions of such hybrids. Åkerman (1921) reported dwarf and crippled hybrids between E. hirsutum and E.montanum, but he did not find many reciprocal differences (cf. Compton 1913a and b). Geith (1924), on the other hand, found malformations in many hybrids, the reciprocals of which were quite normal or had malformations of different level. In some cases he failed to get any hybrids out of crosses made in one direction because of early death of all the seedlings, while the crosses made in the other direction yielded bybrids which survived and could grow to maturity.

The F2 and/or backcross hybrid progeny show a great variety of forms, ranging from strongly inhibited to uninhibited and from completely sterile to nearly fertile, as a result of segregation. Lehmann (1919) obtained some seeds from F1 *E.palustre x parviflorum* (by selfing?) which germinated. Many seedlings died at different stages, early or late, and only 16 plants grew to maturity. These could be classified into 6 or 7 types, some of them being very distinct. Åkerman (1921) and Renner and Kupper (1921) and others also demonstrated considerable segregation in their backcrosses. Lehmann (1925) reported more instances of segregation in later generation hybrids and reviewed the then situation as to our knowledge of *Epilobium* hybrids. He held that different results sometimes obtained by different workers in the same species hybrids were due to the different strains of the species used in the crosses. He particularly commented on the absence of hybrids between *Chamaenerion* and *Lysimachion* in nature.

Just at this time, Michaelis (1925) became interested in *Epilobium* hybridology. He demonstrated that absence of hybrids between *Chamaenerion* and *Lysimachion* was due to seed-incompatibility. Seed failure occurred when *E.angustifolium* and *E. fleischeri* were crossed to *E.hirsutum*, *E.parviflorum* or *E. montanum*. He carried out both extensive and intensive hybridization between *E.hirsutum* and *E.luteum* and between different strains of *E.hirsutum* and extended his studies of the hybrids over several generations. Through this long series of experiments, Michaelis (1933, 1938, and 1954) established that the different strains of *E.hirsutum* differed in their cytoplasm, and so did *E.hirsutum* and *E.luteum* between themselves. In other words, differences between species or within species were being built up not only in genic contents but also in cytoplasm. He also found in his experiments physical malformations of various degrees and sterility in the hybrids. He attributed these effects to the presence of independent cytoplasmic particles, the plasmagenes, in the cytoplasm, which were maintained and inherited independently of the nuclear genes.

Lehmann (1932, 1936, 1941a and b; Lehmann and Duppel, 1950), who had continued his studies of *Epilobium* hybrids with equal fervour, did not agree with Michaelis in the latter's categorical statement about the independent existence and inheritance of the plasmagenes. Lehmann saw physical abnormalities of the hybrids in the light of physiological disturbances, lack of growth hormones, caused by 'inhibitory genes'. Later, when the constancy of the cytoplasm was proved, Lehmann modified his stand by ascribing to the cytoplasmic products of the nuclear (inhibitory) genes the ability of self-reproduction like virus. He also demonstrated segregation of these genes. Full details of their views and works can be found in Caspari (1948), Lehmann and Duppel (1950), and Michaelis (1954).

Some of the experiments of Michaelis (1940a and b) involving interstrain crosses of E.hirsutum are very significant in the sense that they show up inhibitions to the same extent, or even more, as seen in interspecific crosses. Thus great caution must be exercised in measuring differences between species on this basis.

From what we have now seen, we can safely conclude that most of the European species of the Sect. Lysimachion directly or indirectly hybridize but that the Sect. Chamaenerion and the Sect. Lysimachion cannot be hybridized together. The (Lysimachion) hybrids exhibit a number of interesting features both in respect to their viability and fertility. The fertility varies a great deal from hybrid to hybrid. Also different hybrids show different degrees of vegetative vigour. Sometimes the reciprocal crosses differ in these characteristics showing cytoplasmic differences. How far cytoplasmic differences can be a measure of specific divergence is highly doubtful especially when intraspecific hybrids (mostly of the species of *Schizostigma*) sometimes reveal the same type of differences.

TECHNIQUE OF BREEDING

All plants used for artificial pollination were grown in an insect-proof greenhouse. The right size of bud for emasculation was chosen according to the weather conditions. Dehiscing anthers were directly brought into contact with the stigma of the emasculated flower and dusted over it. Where economy of pollen had to be exercised, pollination was affected by a sterilized fine camel hair brush.

Where emasculation was essential before opening of the flower, the petals were removed and the stamens were taken out very carefully taking every precaution not to damage the carpel (style and stigma).

In descriptions, which follow, the first species mentioned in a combination is the female parent.

SEED - INCOMPATIBILITY

Method:-

For a comprehensive and detailed study of development of a normal seed in E. montanum, both paraffin method (microtome sections) and Bradley's (1948) squash technique were employed. The squash technique proved more convenient and was consequently adopted throughout the present work. It had some obvious advantages. Intact embryo sacs containing both embryos and endosperm at the same time could readily be obtained, rather remarkably well during early stages of the seed development. Secondly, it facilitates quick examination of more ovules in less time. The technique was originally developed by Bradley for embryo sacs of Nicotiana, Petunia, and a few other genera to study megagametogenesis and early stages of fertilization. It was described as being particularly useful for genera having numerous small ovules. With a slight modification, it worked well in Epilobium.

The procedure followed in the present study runs as below: (i) Pollinated capsules (cut into small pieces) of different ages were fixed in 1 : 3 acetic-alcohol and stored in the deep -freeze for 3 - 5 months. In fact, storage in the deep-freeze is by no means essential and a 24-hour fixation is sufficient. Nevertheless, storage does not spoil the material and is rather helpful if a large amount of material is to be collected during one flowering and fruiting season.

(ii) Capsules were drained off the fixative, washed in water and placed in a small tube containing a very dilute solution of ferric-chloride instead of 4% aqueous iron alum solution which Bradley used. In the case of Epilobium, the use of ferric-chloride solution as mordant was a definite improvement over the iron alum solution in terms of better differentiation between cytoplasm and nucleus.

(iii) The tube with pieces of capsules in the ferric-chloride solution and two other tubes containing distilled water were put in a water bath at 60°C. After 4-5 minutes, the capsules were transferred from ferric-chloride solution to one of the tubes containing distilled water and then to the other, keeping in each for 3 minutes. The whole operation lasted for 10-11 minutes in the bath. A longer treatment at a lower temperature (cf. Bradley's 7 minutes treatment at 75°C) does not damage the non-cellular, and hence very delicate, endosperm in Epilobium.

(iv) The tube of ovaries was removed from the bath and cold water was poured into it. Ovaries were left in cold water for 2-3 minutes.

(v) Ovaries were macerated in 10N HCl for 10 minutes at room temperature.

(vi) Then they were washed in several changes of distilled water, and kept at least for 20 minutes in water altogether.

(vii) A piece of the capsule was taken out and ovules were scraped from the placenta in a drop of aceto-carmine on a slide. All bits of tissue other than ovules were removed.

(viii) Ovules were tapped gently under cover-slip, heated over a mild flame and squashed under pressure of thumb. Embryo sacs came out easily of the ovules and were stained well in aceto-carmine. Empty ovules had nothing inside them. All preparations were examined under the miscroscope.

(ix) Preparations were made permanent by running aquamount solution under cover-slip and allowing it to dry.

<u>A mature embryo sac in Epilobium</u>:

A mature embryo sac in *Epilobium* is four-nucleate, consisting of an egg, two synergids and a polar nucleus. There are no antipodals; nor is the polar nucleus formed as a result of fusion between two nuclei from each pole. All the four nuclei are derived from only one of the megaspores; three others disintegrate. Hence it is also called monosporic, the details of which can be had from a description of the *Oenothera* type of embryo sac in Maheshwari (1950). The significance of such a constitution of the embryo sac lies in the fact that the polar nucleus is haploid on a par with the egg and that both the zygote and the primary endosperm nucleus become disloid after fertilization. In most other angiosperms, the primary endosperm nucleus is triploid and the zygote diploid.

Course of development of a typical embryo in Epilobium:

The embryo in Epilobium is of the Onagrad type (Maheshwari, 1950; Johansen, 1950; and Wardlaw, 1955) and a detailed account of its development is to be found in Johansen (1950, pp. 167-169).

For our purpose, it was considered desirable to select one of the species frequently used in the present hybridization experiments, to study step by step all the stages of development of its embryo as it normally happens without artificial pollination, and to compare with it the development of the hybrid embryos. E. montanum L. was accordingly chosen.

Figures 3 and 4 show all the important stages of growth of an embryo in E. montanum in a sequence from an one-celled zygote to a complex and fully differentiated embryo in a mature seed. They also show, side by side, phases of organization and disintegration of its contemporary endosperm.

EMBRYO:- The first division of the zygote is always transverse differentiating two cells, an apical cell (ca) and a basal one (cb) (Fig. 3b). This is followed simultaneously by another transverse division of the basal cell and a longitudinal one of the apical cell. The apical cell forms the embryo propoer and the basal cell goes to form the hypophysis and the sus-

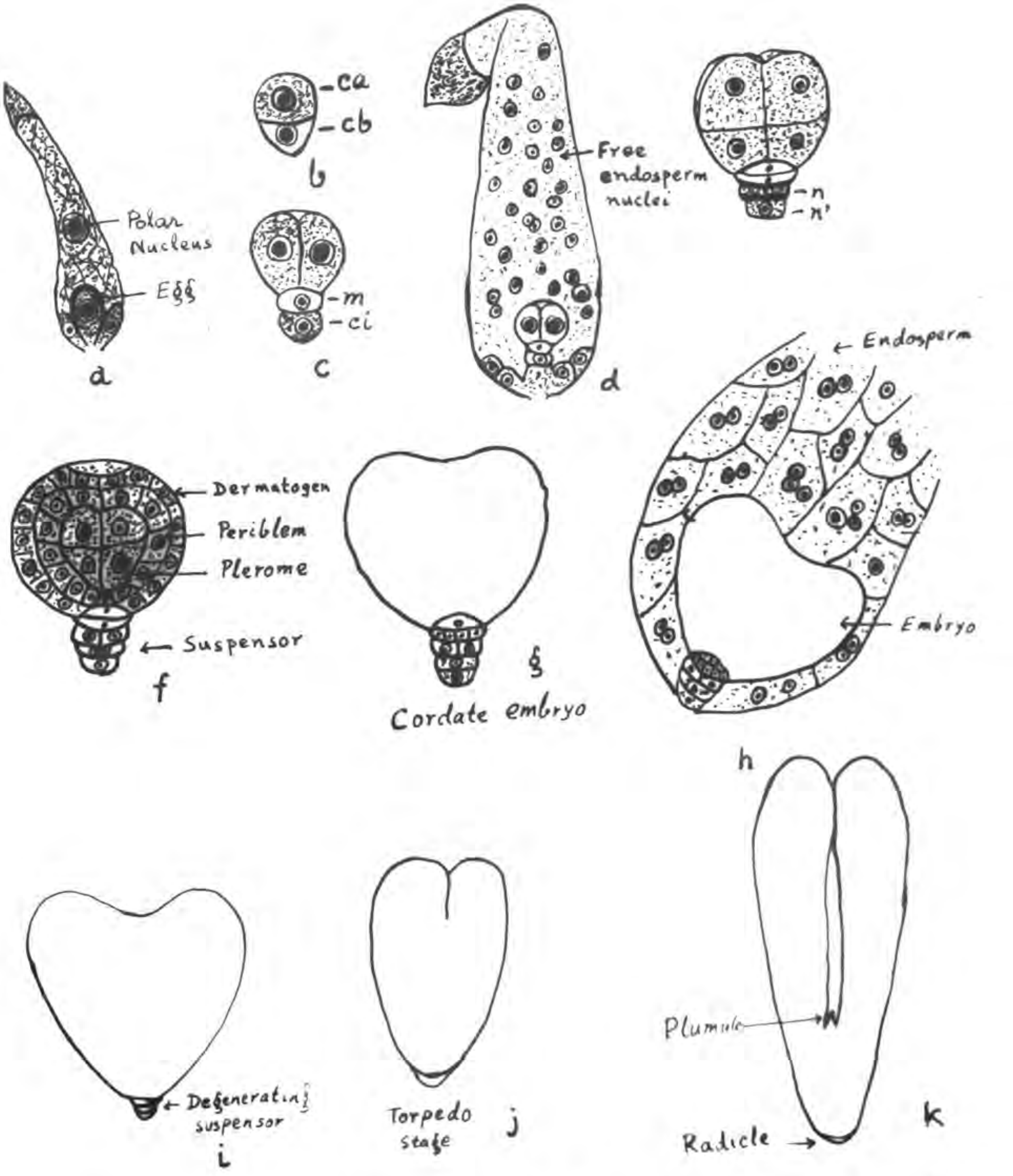


Fig 3 a-k. Diagrams showing embryo development in *E. montanum*. See text. a-i (except d) ×100; d ×50, j&k ×50.

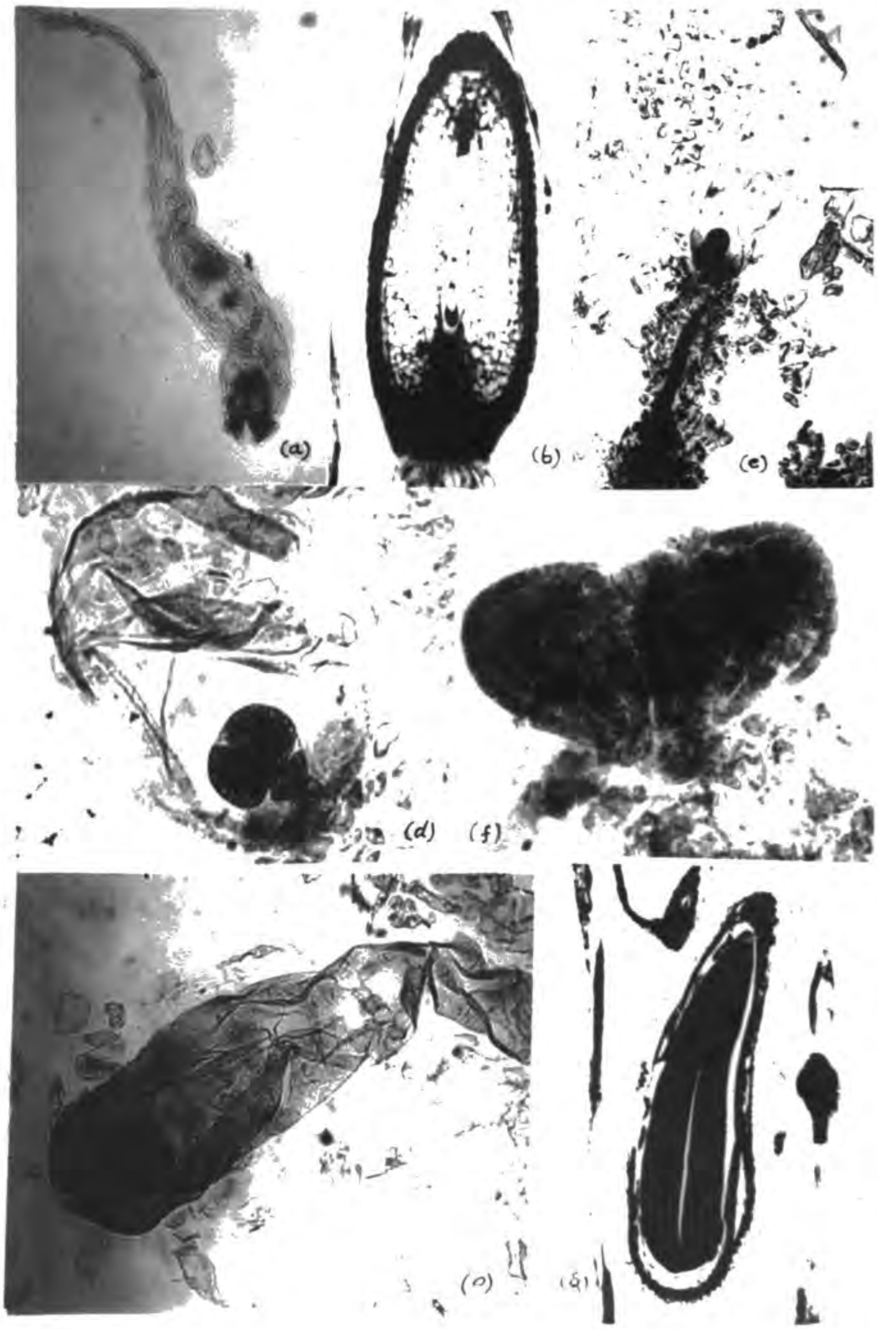


Fig. 4. Photographs of different stages of seed development in *E. montanum*.

(a) embryo sac, × 350 ; (b) young embryo, a few endosperm nuclei, × 200 ; (c-e) embryos in different stages of growth, note endosperm nuclei dividing in e, × 250 ; (f) cordate embryo with no endosperm, × 500 ;

(g) mature seed, × 50.

pensor.

<u>Apical cell</u> or <u>ca</u>:- The next division of the apical cell is again longitudinal at right angles to the first one, thus forming the four-celled stage (quadrant) of the embryo (Fig. 3c). In immediate succession to this, a third division, which is transverse, differentiates the embryo into an octant stage. (Fig. ec). The upper tier of four cells of the eight-celled embryo gives rise to cotyledons and the lower tier eventually becomes the hypocotyledonary region. Sometimes, although very rarely, the second division of the apical cell is transverse and the third one longitudinal instead of being the other way round. In any case, normal differentiation of the octant stage of the embryo is attained without any disturbance. The two divisions in fact are so quick in succession that it is hard to determine the precise sequence.

Soon after the octant stage has been attained, periclinal division initiates three germinal layers, the dermatogen, the periblem, and the plerome (Fig. 3f). Very soon a heart-shaped embryo (Fig. 3g) is differentiated with active growth in the cotyledonary portion. Rapid growth of the cotyledons gives embryo a characteristic shape called "torpedo" because of its resemblance to a torpedo (Fig. 3j). The mature embryo (Fig. 3k) consits of cotyledons for more than two-thirds of its entire length; these are flattened against each other, protecting the plumule in the notch.

Basal cell or cb: The fate of the two cells derived by the first transverse division of the basal cell (Fig. 3c) is different. The upper daughter cell m forms the hypophysis and gives rise to the root tissues and the root cap. The lower cell, ci, forms the suspensor.

Both the shape and division of m are very characteristic. The upper wall of m adjoining the apical cell of the embryo pushes upward into the embryo, looking like a curved roof, and then a transverse division cuts off an upper lens-like cell and a lower watch-glass like cell (Fig. 3e - f). The upper lens-like cell is very slow to divide further and remains undivided for a long time. The lower cell, on the other hand, divides vertically very quickly into four cells (Fig. 3g). Afterwards, the upper lens-like cell also divides vertically into four cells. Thus two groups of four cells each are derived from m; the derivatives of the upper daughter cell of m forming the root-cortex and those of the lower one the root-cap (Fig. 3h - k).

The lower daughter cell ci of the basal cell cb devides transversely into two cells, n and n' (Fig. 3e - f). Cell n does not ordinarily divide again, but cell n' usually divides transversely and thus a three-celled suspensor (very rarely four-celled due to a longitudinal division in n)is formed. The suspensor begins to degenerate quickly after the cordate stage of the embryo (Fig. 3i).

ENDOSPERM:- Division in the primary endosperm nucleus starts earlier and quicker than in the zygote. Figure 5 shows a comparison between developments of the two contemporary tissues, the embryo and the endosperm. By the time the quadrant stage of the embryo is reached, free nuclei of endosperm have been dispersed evenly throughout the entire sac. - (Fig. 3d). As the embryo-sac enlarges gradually, more endosperm nuclei are produced, and because the activity of cell-wall formation has been avoided, the rate of the production of free nuclei is faster. Cellular appearance of the endosperm is visible sometime after the cordate stage of the embryo (Fig. 3h) and two to three or more nuclei may be found included in a cell. Soon after, the endosperm hardens and disintegrates. The mature embryo, which is now like a pair of forceps, comes directly in contact with the seed-coat, the nucellus being already disorganized with the growth of the embryo and the endosperm.

Table 6 gives a summary of the developmental stages at different periods of growth.

With this information in hand, we shall now examine the interspecific hybrid embryos at all stages of their development

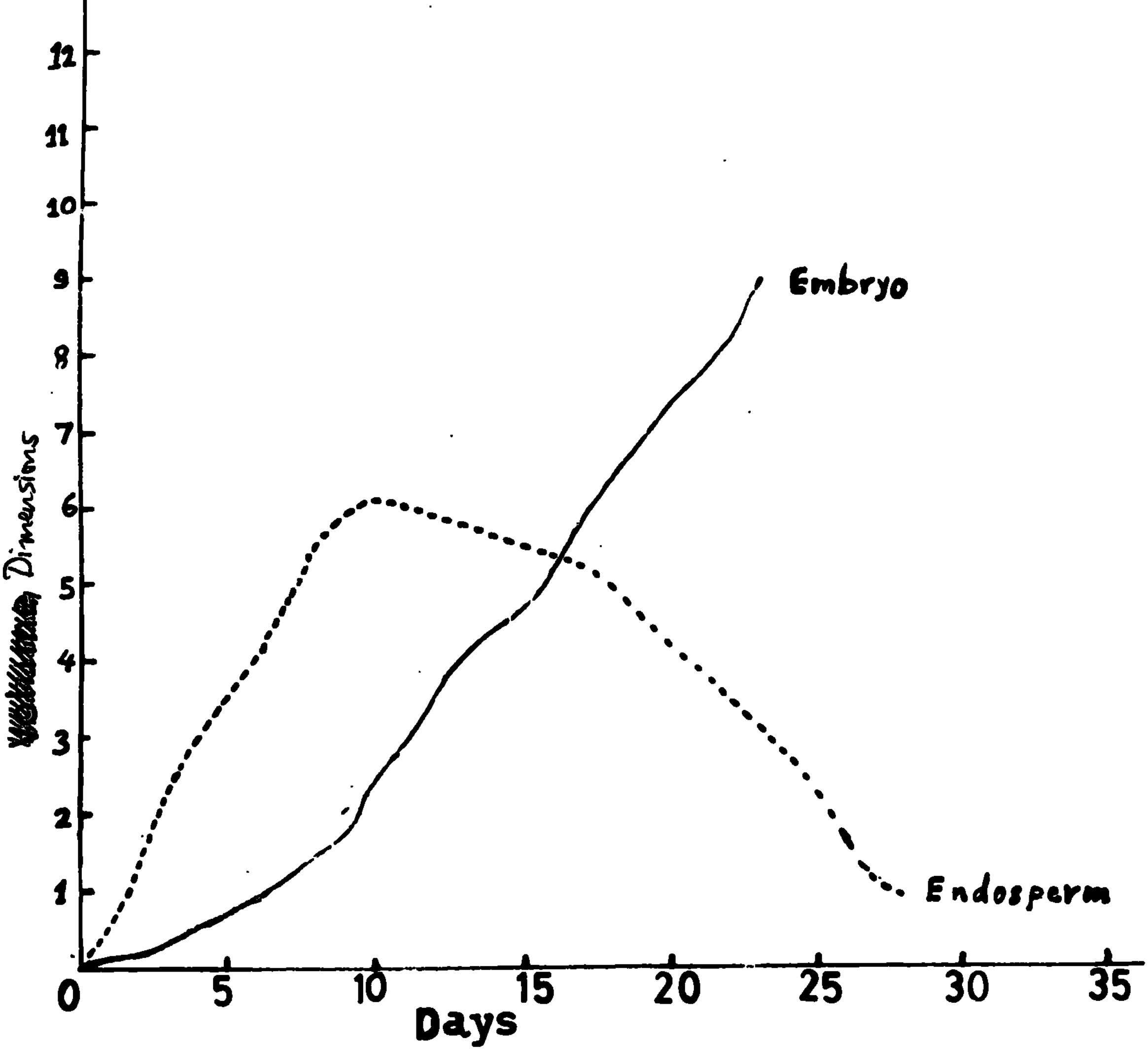


Fig. 5. Graph showing increase in ~~volume~~ dimensions of embryo and endosperm of a developing seed of E. montanum. (Dimensions in arbitrary units.)

TABLE 6

A summary of all the important stages in development of seed in E. montanum

Days after pollination	Stage of embryo	Description
24 hours	-	Fertilization takes place, but the zygote does not divide.
36-48 hours	2-celled pro-embryo	The apical and the basal cells formed.
3 days	Octant stage	Numerous endosperm nuclei; cell m of the basal divides into an upper lens like and a lower watch-glass like cell; ci also divides transversely into two.
5-6 days	*Reversed trapezium	Height and breadth of embryo equal; three germinal layers differentiated; endosperm actively dividing.
8-9 days	Cordate	Suspensor has fully developed and now begins to degenerate; endosperm cellular.
12-15 days	*Torpedo	Cotyledons become flattened against each other and form half the length of the embryo; endosperm rapidly disorganizing; suspensor may or may not be seen; nucellus disorganized.
20 days	Mature embryo	With more than two-thirds of the length consisting of the flat cotyledons; endosperm and nucellus entirely disorganized.
The capsule bursts open with mature seeds within 25-30 days.		

*Terms adopted from Wardlaw (1955)

and see whether

(i) the cells are cut off from the zygote and laid down in the established sequence and with regularity;

(ii) the development of the embryo, the suspensor and the endosperm is balanced and proportional;

(iii) the disorganization of endosperm and nucellus (or even of suspensor) is initiated at the right stage so as not to cause damage to the growing embryo; and

(iv) no abnormal tissue is found developing within the seed.

INTERSECTIONAL CROSSES: CHAMAENERION X LYSIMACHION (SCHIZOSTIGMA)

A summary of the crosses made is given in Table 7.

The number of capsules in the Table 7 does not include those which were fixed for the study of seed development after pollination. It will be noted that no perfect seed was obtained in any of the crosses involving E. angustifolium. However, shrunken seeds of various size were present inside the capsules. Quite a few of them upon dissection, were found to contain minute embryos which had aborted. But most of the seeds were empty. Empty or not, all seeds had a well-developed bunch of hairs.

In crosses involving E. fleischeri, on the other hand, a

TABLE 7

A summary of the crosses made between Chamaenerion and Lysimachion

Cross	No. of capsules pollinated	Results
1. E. angustifolium x hirsutum		
P16762 x KR5862	13	Cross failed or seeds abortive
60.31 Force Burn x KR5862	4	"
2. E. hirsutum x angustifolium		
KR5862 x P16762	4	"
KR5862 x 60.31 Force Burn	5	"
672 x P16762	4	"
3. E. angustifolium x parviflorum		
P16762 x SP6262	18	"
60.31 Force Burn x SP6262	8	"
4. E. parviflorum x angustifolium		
SP6262 x P16762	11	"
SP6262 x 60.31 Force Burn	14	"
5. E. angustifolium x montanum		
P16762 x PL63	6	"
P16762 x SL621	5	"
60.31 Force Burn x SL621	13	"
6. E. montanum x angustifolium		
PL63 x P16762	9	"
SL621 x P16762	3	"
SL621 x 60.31 Force Burn	13	"
616 x 60.31 Force Burn	4	"
7. E. fleischeri x luteum		
U62 x U261	5	Only 5 apparently good looking seeds.
8. E. luteum x fleischeri		
U261 x U62	4	" 6 " "
9. E. fleischeri x hirsutum 672	5	" 4 " "

few good looking seeds, 1 or 2 per capsule, were always found and there was a wide range of variation in size of the seeds from those containing nothing inside them to those approaching 'normal size' of a seed. None of the seeds of E.fleischeri and E.luteum cross in either direction germinated, while two obtained from E.fleischeri x hirsutum cross did.

All evidence suggests that fertilization takes place but the embryos fail to grow to maturity; they abort at different stages of their development. Developing ovules of only one hybrid combination involving E.fleischeri have been examined; but on the basis of this and the size of seed attained usually in other combinations, it is evident that hybrid embryos involving E.fleischeri grow bigger and fail at a later stage as compared to those involving E.angustifolium.

Detailed investigation of seed development has been made in the following crosses:

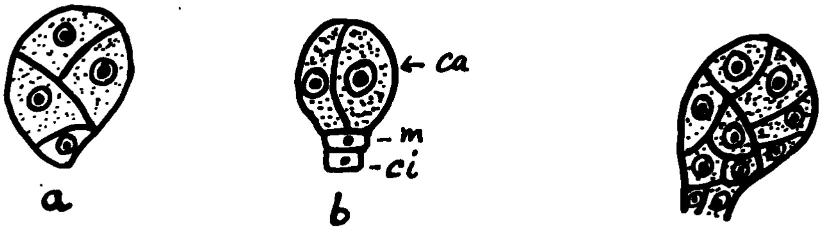
1. E.angustifolium x hirsutum

Embryo: Practically no dividing zygote was found in ovules fixed 2 days after pollination. Only in a few cases, always in ovules scraped from the upper part of the capsule, was the proembryo seen to divide. Capsules fixed 3-4 days after pollination had ovules with embryos mostly at quadrant stage. The division is regular, but as compared to that in normal seed development in E.montanum it is very slow. Embryos degenerate quickly after this quadrant stage. All ovules examined after 6-12 days of pollination revealed nothing beyond the quadrant stage of the embryo. They were all empty because the embryos had already degenerated. Some of the ovules gave out some sort of a mass or lump of tissue under squash this was probably an undifferentiated embryo without definite cotyledonary and suspensorial regions.

Endosperm: Endosperm development is slow from the very start. The rate of division of the endosperm nuclei is too slow with the result that very few nuclei are seen in the embryo sac with an embryo at quadrant stage. This retards the growth of the pro-embryo which collapses after the quadrant stage probably due to starvation.

2. E.hirsutum x angustifolium

Embryo: Division in the zygote and the early embryo is mostly regular, but in nearly one third of the cases the pro-embryo begins to divide irregularly. The first division in the basal cell is oblique rather than transverse; thus there is no satisfactory differentiation of m and ci of the basal cell (Fig. 6a). Pro-embryos with oblique division in their basal cells start to cut off cells more in one dimension than in others, (Fig. 6c) and thereby the polarity of the growing embryo is disturbed. A very irregular mass of embryo is formed (Fig. 6e). Germinal layers cannot be differentiated nor can one mark out



a
Irregular division in Pro-embryo

b
Regular division in Pro-embryo

c.
Unbalanced division

d
Embryo-sac containing degenerating endosperm and an undifferentiated embryo.

e
An undifferentiated embryo.

Fig. 6. Seed failure in E. hirsutum x angustifolium cross.
All magnifications x c. 100.

the suspensor. Only a faint impression of what might be a cotyledonary area can be had.

Pro-embryos dividing regularly do so until the octant stage is reached. Soon after, irregular division commences and ultimately an undifferentiated embryo is produced.

Endosperm: Endosperm begins to degenerate much quicker before the cordate stage of the embryo is attained. 2-3 or more nuclei are included in an irregular cell and further production of free nuclei is limited (Fig. 6d).

3. E.angustifolium x parviflorum

Embryo: Contents of the embryo-sacs degenerate quite early. More than half of the ovules collected 4 days after pollination showed that their embryos had developed to the octant stage. No abnormality in early division of the pro-embryo was detected. All ovules fixed after 6 days of pollination had degenerated embryos. Some of them under pressure released an irregular minute mass of tissue, the undifferentiated embryo.

Endosperm: Endosperm is scanty and never more than a few free nuclei are seen at any stage of development. It disintegrates much before the embryo.

4. E.parviflorum x angustifolium

Embryo: Early divisions in the pro-embryo are regular. It is fairly easy to trace the development till the octant stage. Thereafter, irregular division takes place obscuring proper differentiation of the embryo and it is the undifferentiated embryo which comes out under pressure from many of the ovules. But in majority of the ovules, the growth of the embryo seems to cease very early.

Endosperm: Division in the primary endosperm nuclei is much delayed and only a few endosperm nuclei are seen along with the quadrant stage of the embryo. This low rate of production of free nuclei continues only till the octant stage of the embryo is reached and thereafter the endosperm quickly disintegrates.

5. E.angustifolium x montanum

Embryo: Embryos degenerate very early in their development. A few of them which survive grow into an undifferentiated mass of tissue. Right up to the quadrant stage, the development has been traced to be regular. Irregular division starts after that and at the cordate stage some of the embryos can be seen to have inequal cotyledonary lobes. Most of the embryos degenerate before reaching this stage.

Endosperm: The fate of the endosperm is almost the same as we have seen in the earlier cases. The division of the endosperm nuclei is very slow and it is disorganized very soon.

6. E.montanum x angustifolium

Embryo: In most of the ovules, embryos develop to the octant stage without any disturbance. Later, nearly half of them begin to divide very slowly and ultimately die. The rest of them divide irregularly and it is hard to find out how the cells are laid down. The result is the same; an undifferentiated embryo is produced.

Endosperm: Disorganization of endosperm with slowing down of the rate of free nuclei division commences on the same pattern as it occurs in the above crosses.

7. E.fleischeri x hirsutum

Embryo: Division is regular in early stages of development of the embryos. It is only after the octant stage that abnormal growth begins. However, a few of them still reach the cordate stage quite normally but the division is considerably slow. Embryos undergoing irregular divisions die at various stages, while some of them attain a large size although remaining undifferentiated.

Endosperm: The rate of division of free endosperm nuclei is slow but it survives until the cordate stage of the embryo, after which it quickly disintegrates.

INTRA-SECTIONAL, CROSSES: SCHIZOSTIGMA X SCHIZOSTIGMA

Crosses made are summarized in Table 8.

Percentage of seed set in a cross has been calculated on the basis of the mean of the total number of seeds obtained in all the capsules pollinated as compared to the average seed set

TABLE 8

A summary of the crosses made between Schizostigma species

Cross	No. of capsules pollinated	Approx. % seed set	Approx. % Germination
1. E. hirsutum x parviflorum			
KR5862 x SP6262	7	25-30	70
NC1 x 163	9	25	85
2. E. parviflorum x hirsutum			
SP6262 x KR5862	8	22	33
675 x KR5862	7	35	35
3. E. hirsutum x montanum			
KR5862 x PL63	11	30	50-55
KR5862 x SL621	5	25	55
672 x 616	7	15	60
NC1 x 616	4	40	45
4. E. montanum x hirsutum			
SL621 x KR5862	7	35	45
PL63 x KR5862	3	25	33
5. E. hirsutum x luteum			
KR5862 x U261	9	25	80
672 x U261	11	33	76
NC1 x U261	8	30	84
6. E. luteum x hirsutum			
U261 x KR5862	5	35	74
U261 x 672	4	33	80
7. E. parviflorum x montanum			
SP6262 x SL621	6	40	70-75
675 x 616	7	45	80
8. E. montanum x parviflorum			
SL621 x SP6262	11	43	84
PL63 x 620	3	35	76
616 x SP6262	5	48	80
9. E. parviflorum x luteum			
SP6262 x U261	7	35	70
10. E. luteum x parviflorum			
U261 x SP6262	6	48	65
11. E. montanum x luteum			
SL621 x U261	9	37	78
616 x U261	13	46	75
12. E. luteum x montanum			
U261 x SL621	6	40	66
U261 x 616	9	40	70
13. E. lanceolatum x parviflorum			
AFW604 x 620	6	65	70
14. E. lanceolatum x luteum			
AFW604 x U261	9	67	80
15. E. montanum x lanceolatum			
PL63 x AFW604	13	55	63
16. E. lanceolatum x montanum			
AFW604 x PL63	8	60	65
AFW604 x 617	7	65	73

per capsule in the female parent in nature. It must be emphasized that seed set in different capsules in the same cross was sometimes very different, depending perhaps on fluctuations in weather and other conditions such as the amount of good or bad pollen dusted on the stigma.

There is a fair amount of seed set in almost all the Schizostigma x Schizostigma crosses. It is quite high (above 55%) in crosses involving E.lanceolatum with E.parviflorum, montanum and luteum. E.parviflorum and E.montanum, crossed in either direction, also set a high amount of hybrid seed between themselves. Equally good amount of seed is set in the crosses involving E.luteum with E.parviflorum or E.montanum in both directions. However, all crosses involving E.hirsutum appear to result in low seed set.

Ordinarily, the germination of these hybrid seeds is very good. Surprisingly enough, there is a considerable difference in germination of seeds of the reciprocal crosses between E. hirsutum and E.parviflorum. Merely coming out of the cotyledons and breaking open the seed coat has been counted as germination and it does not take into account further growth of the seedlings.

Detailed investigation of seed development has been made in the following crosses:

1. E.hirsutum x parviflorum

Well-developed seeds are usually confined to the upper part of the capsule, but a few of them can also be found in the lower portion. Whether fully developed or not, all seeds have a normal bunch of hairs.

Embryo: Early divisions in the pro-embryo are regular, ovules fixed 3 days after pollination produce embryos at the octant stage. Thereafter the rate of division in different embryos seems to be different in the same capsule, some dividing slowly while others grow faster. This is shown by the presence of embryos of different sizes and at different stages of their growth in the same capsule. The lower the position of ovules in the capsule, the slower is the rate of growth in their embryos. Less than half the total number of ovules fixed 8 days after their pollination were found to contain cordate embryos; more than 75% of these were confined to the upper half of the capsule. In the lower region of the capsule, only a few cordate embryos were found. Embryos which fail to reach the cordate stage within a certain time limit, usually within 8 to 10 days after pollination, eventually stop growth and degenerate. Ovules fixed 15 days after pollination had either 'torpedo' embryos or decaying ones hardly recognizable into any shape. The 'torpedo' embryos grow into mature ones, but it appears that a few of them do not develop to perfect maturity as is evidenced by seeds of smaller sizes.

Endosperm: Practically no abnormality has been detected in the endosperm growth. The primary endosperm nucleus starts dividing earlier than the zygote and there is no indication that the rate of free nuclear division of endosperm is by any means slower than the rate of division in the contemporary embryo. Fewer nuclei are seen in the embryo sacs containing slow growing embryos and it is the endosperm which degenerates before the failing embryos, yet it is hard to tell which of the two grows more slowly so as to inhibit growth of the other. At any rate endosperm begins to disintegrate after the cordate stage of the embryo is attined. The endosperm by this time is cellular in appearance.

2. E.parviflorum x hirsutum

Nearly 50% of the seeds are empty, 20% are imperfectly developed, and about 25-30% of the seeds are well-developed with mature embryos. As in the reciprocal, good seeds are more abundant in the upper region of the capsule. Imperfect seeds contain minute dried embryos which fail somehow or other to develop to maturity at a very late stage. There are some seeds, usually 5 to 6 per capsule, unusually big in zise among the well-developed ones (Fig. 7) and these germinate more quickly than the rest.

Embryo: Development appears to be normal, but the failing

Fig. 7. Photograph of hybrid seeds of E.parviflorum x hirsutum. Note at least 5 different sizes. x 60.

embryos are slow to divide and embryos at different stages of their growth can be seen in the same capsule. 50% of the embryos fail to develop before the cordate stage. The rest continue to develop, some failing midway between the cordate and the torpedo stage, and a majority of them attaining maturity, no anomaly in development has been detected but the slow rate with which they develop is perhaps an indication of lack of some sort of growth stimulus embryos which fail to develop before attaining the cordate stage seem to degenerate very quickly and the seeds become empty.

Endosperm: The endosperm is very slow to undergo free nuclear division. It is disorganized before the cordate stage of the embryo is reached in all the failing seeds. It hardly survives beyond this stage. Of all the preparation examined, only 5 embryo-sacs intact with endosperm and cordate embryo were observed.

3. E.hirsutum x montanum

Good seeds are contained mostly in the upper half of the capsule. Nearly 50% of the seeds are empty, while 25-30% of the seeds contain embryos of various size and without much differentiation (Fig. 8a and b).

Embryo: After fertilization, the zygote proceeds to divide quite normally and the division is quite regular till the embryo

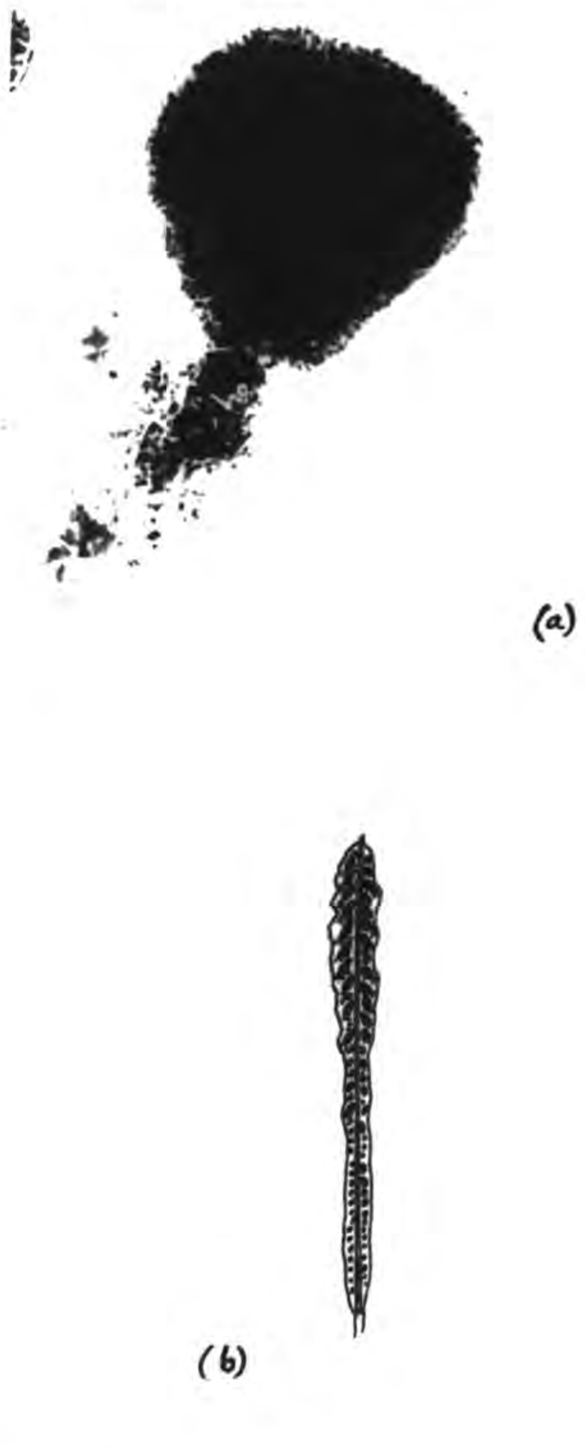


Fig 8. E. hirsutum x montanum. (a) Photograph of an undifferentiated and dying embryo. x 500.
(b) Diagram (drawn from actual specimen) of a capsule containing good seeds in the upper part which is swollen.

passes much beyond the octant stage, somewhere near the 'reversed trapezium' stage. After this the rate of division in different embryos of the same capsule appears to be different with the result that embryos of different size are found in the same capsule. Not only is the rate of division in the failing embryos slow but also the division is irregular. It must be added here that most of the ovules in the lowermost part of the capsule do not appear to have been fertilized because no dividing embryo is usually obtained in any of the preparations. Embryos dividing irregularly become undifferentiated; most of them degenerate at various stages. Embryos which get differentiated quickly into the cordate stage most often develop to maturity; although a few of them still fail to grow to perfection.

Endosperm: The endosperm degenerates very early. Production of free endosperm nuclei is meagre and before the cordate stage of the embryo is reached the endosperm has already disintegrated.

4. E.montanum x hirsutum

Perfect seeds are confined to the upper region of the capsule. Imperfect seeds are of different size, either completely empty or containing undifferentiated embryos.

Embryo: Initial development is regular, right up to the pre-cordate stage. Nearly 25% of the embryos get differentiated into the cordate stage and develop to maturity, whereas the rest begin to grow very slow and fail to attain maturity. No abnormality in development of the failing embryos (*cf*. the reciprocal cross) could definitely be traced.

Endosperm: Dissolution of endosperm is very quick and is completed before the cordate stage of the embryo in all the failing seeds.

5. *E.hirsutum x luteum*

Embryo: The rate of growth of embryos in the same capsule is different; the lowermost part of the capsule usually containing ovules with no diving embryos. The development has been traced to be normal right up to the full differentiation of the embryo into a mature seed. Slow developing embryos fail and degenerate at various stages of their development.

Endosperm: Endosperm growth is normal; the nuclei divide much faster than the slow growing embryos destined to collapse. The endosperm degenerates after reaching its maximum growth and with it degenerates the slow growing embryo, which is unable to survive without endosperm because of its relative immaturity. (Fig. 9).

6. *E.luteum x hirsutum*

Embryo: As in the reciprocal cross, there are slow growing embryos which fail to develop at various stages and there are embryos growing with normal,rate which finally attain maturity.

Endosperm: Endosperm growth is normal both with the slow-

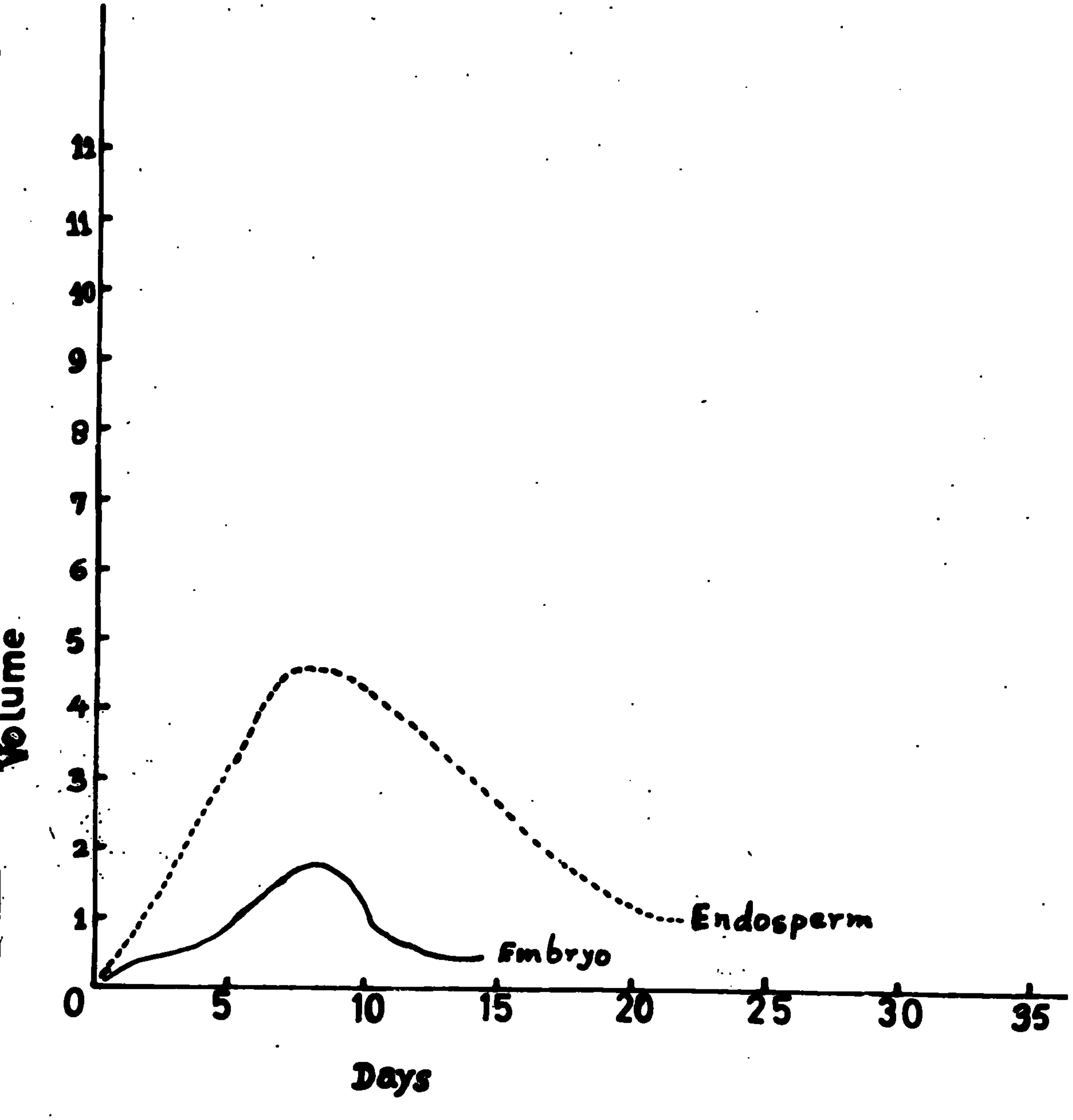


Fig. 9. Graph showing increase in volume (in arbitrary units) of embryo and endosperm in a developing hybrid seed of <u>E. hirsutum</u> x <u>luteum</u>.

growing as well as the normal embryo.

7. E.parviflorum x montanum and 8. the reciprocal:

Percentage of seed set is fairly good in both the crosses. Good seeds are evenly dispersed throughout the length of the capsule, though empty seeds are mostly found in the lowermost region.

Embryo: Divisions in the embryo are pretty regular and all embryos attain the cordate stage with equal efficiency. A few of the ovules in all preparations never seem to contain dividing embryos; such ovules are concentrated in the lowermost part of the capsule. Some of the cordate embryos fail to grow further, while most of them grow and reach maturity. Why failure occurs in some of the embryos is not very clear. But in a few cases, it has been observed that the synergids are quite active and divide to 2-3 celled stage with the young embryo. Also some extra-cells, perhaps proleferating ours, are seen.

Endosperm: Division in the endosperm nuclei is also normal and it degenerates only after the cordate stage of the embryo is attained following perfectly a normal course. But in the failing seed, the rate of the endosperm growth appears to be inhibited.

9. E.montanum x luteum

Embryo: Rate of development of embryos in the same capsule is different after the octant stage. All ovules fixed after 3 days of pollination contain embryos of the octant stage. Most of the embryos (Cf. E.hirsutum x luteum) get differentiated into the cordate stage and the rest struggle and fail at various stages in between the octant and the cordate stage. All embryos passing through the cordate stage attain maturity. The developmental course is normal throughout.

Endosperm: Growth of endosperm is in all cases normal.

INTRASECTIONAL CROSSES: SCHIZOSTIGMA X SYNSTIGMA

Table 9 gives a summary of all the crosses made in this section.

It is evident from the table that seed set is low in crosses involving E.hirsutum and in 3 other crosses involving E.parviflorum with E.tetragonum and E.palustre.

The crosses between E.montanum and a Synstigma species appear to yield a high percentage of seed, except the cross E.montanum x nerterioides and the reciprocal which did not succeed and the crosses between E.montanum and E.Lamyi which resulted into 30-35% seed set. The crosses between E.parviflorum and E.obscurum and between E.parviflorum and E.Lamyi also result in a very high seed set.

Detailed investigation of seed development has been made in the following crosses:

TABLE 9

A summary of the crosses made between Schizostigma and Synstigma

Cross	No. of capsules pollinated	% Seed set	% Germination
1. *E. hirsutum* x *roseum*			
672 x 621 Skåne	5	26	45
NC1 x 621 Skåne	3	60 (all very small in size)	71
2. Reciprocal			
621 Skåne x 672	5	15	52
3. *E. hirsutum* x *palustre*			
KR5862 x 618	4	15	80
4. *E. Lamyi* x *hirsutum*			
15 x NC1	6	12-15	85
5. *E. parviflorum* x *roseum*			
620 x 621 Skåne	7	45	90
6. Reciprocal	6	40	85
7. *E. parviflorum* x *Lamyi*			
620 x 15	20	57	85
8. Reciprocal	8	40	80
9. *E. parviflorum* x *tetragonum*			
620 x PS163	4	25	75
10. *E. parviflorum* x *obscurum*			
620 x Pn763	5	55	90
11. Reciprocal	6	50	87
12. *E. parviflorum* x *palustre*			
675 Leipzig x 619	10	25	80
13. Reciprocal	9	30	78
14. *E. parviflorum* x *adenocaulon*			
SP6262 x SL	7	40	92
15. Reciprocal	7	45	90
16. *E. montanum* x *roseum*			
SL621 x 621 Skåne	9	60	85
17. Reciprocal	7	65	80
18. *E. montanum* SL621 x *Lamyi* 15	7	30	80
19. Reciprocal	6	35	83
20. *E. montanum* x *obscurum*			
SL621 x Pn763	8	53	78
21. Reciprocal	6	55	80
22. *E. montanum* SL621 x *adenocaulon* SL	5	60	72
23. Reciprocal	9	55	78
24. *E. montanum* x *alsinifolium*			
PL63 x R1621	7	55	90
SL621 x R1621	8	55-60	85
25. Reciprocal			
R1621 x SL621	11	60	85
26. *E. montanum* x *nerterioides*			
SL621 x Scot.	7	Cross failed	-
27. Reciprocal	5	"	-
28. *E. lanceolatum* x *Lamyi*			
AFW604 x 15	3	40	35
29. *E. lanceolatum* AFW604 x *palustre* 619	3	40	65
30. *E. lanceolatum* x *alsinifolium*			
AFW605 x R1621	4	38	55
31. Reciprocal	5	40	30 ?
32. *E. roseum* x *lanceolatum*			
Skåne 621 x AFW604	7	35	60

1. E.Lamyi x hirsutum:

Embryo: Early divisions in the zygote and the pro-embryo are regular, except that the lower daughter cell ci of the basal cell cb divides transversely twice. This causes the suspensor to be a bit more massive (Fig. 10a) than usual. No other abnormal tissue is formed. Most of the embryos collapse before reaching the cordate stage.

Endosperm: Endosperm is very slow to divide and degenerates quite early.

2. E.parviflorum x roseum:

Embryo: Some ovules do not seem to be fertilized. No dividing embryo is ever found in their embryo sacs. First two divisions of the pro-embryo are quick and normal. Afterwards the division is extremely slow. After many days, only 2-celled, very rarely quadrant, embryos are seen. These slow growing embryos degenerate. In a few cases, synergids were found to have been growing on both sides of the degenerating embryo (Fig. 10b). With the active growth of the synergids, the embryo perhaps gets starved and fails to grow further.

Endosperm: Division in the endosperm is normal and faster as compared with that in the embryo. But in the failing seeds, in which the synergids are found to be actively dividing, the endosperm growth is very slow.

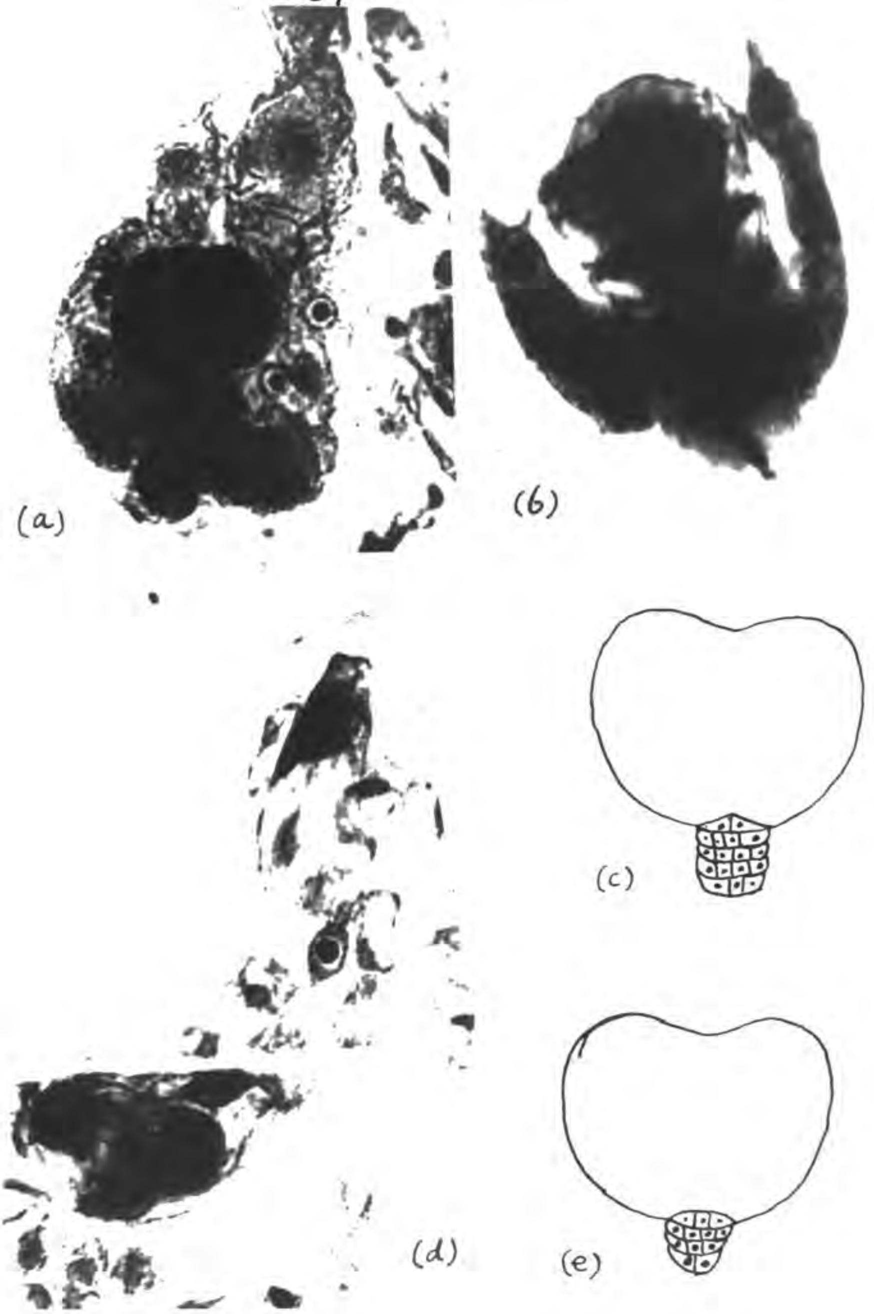


Fig. 10. Some abnormalities in seed development.
(a) Hybrid embryo E. Lamyi x hirsutum with unusually big suspensor, x500 ; (b) Actively dividing synergids in E. parviflorum x roseum, note the dying embryo.
(c) cordate embryo of E. obscurum x adenocaulon with relatively bigger suspensor, note the normal cordate embryo in the reciprocal in (e) ; (d) 5-nucleate embryo sac in E. adenocaulon. All x500.

3. E.parviflorum x adenocaulon:

Embryo: Early divisions in the pro-embryos are normal. When the embryos get differentiated into the quadrant stage, some of them begin to slow down in further growth. Different sizes of embryos are seen in the same part of the capsule. Obviously, some of the embryos grow slow, while others grow comparatively faster. The slow growing embryos eventually degenerate.

Endosperm: Endosperm growth is normal and apparently faster than the embryos.

4. E.roseum x montanum:

Embryo: The rate of development is different for different embryos in the same capsule. A few of the ovules do not seem to contain any dividing embryo from the very start. Probably they are unfertilized. Slow growing embryos fail to attain maturity.

Endosperm: Endosperm growth is normal. However, I found in two cases the primary endosperm nucleus just dividing along with the zygote. This should be regarded as a delayed division of the endosperm. But this type of delayed division was not very common.

5. & 6. E.montanum x adenocaulon and the reciprocal:

Embryo: Development of the embryo in both the crosses is normal. Early divisions of the zygote and the pro-embryo are regular and all the embryos divide with equal rate. After the embryos have been differentiated into the octant stage, nearly half of them do not grow further and collapse. In preparations of ovules fixed 5-6 days after pollination, half of the ovules are found to be empty and the rest growing beyond the octant stage. In mature capsules, one finds fully developed seeds and empty seeds side by side in nearly 1:1 ratio.

However, there is difference in maturation period of the seeds from the reciprocal crosses. Seed maturation in E.adenocaulon x montanum is faster than in the reciprocal. This difference is visible only after the cordate stage.

Endosperm: The endosperm growth is normal.

7. & 8. E.montanum x nerterioides and the Reciprocal.

No seed is obtained in either cross. It seems that fertilization does not take place. All ovules fixed 3 days after pollination were without embryos. In some of the, embryo sacs could be seen but containing degenerating egg apparatus.

INTRASECTIONAL CROSSES; SYNSTIGMA X SYNSTIGMA.

A summary of the crosses made is given in Table 10.

Seed set is very high in almost all the crosses, except in five of the crosses involving E.adenocaulon and in E.Lamyi x tetragonum cross. Crosses between E. roseum and E.nerterioides

TABLE 10

A summary of the crosses between Synstigma species

Cross	No. of capsules pollinated	% Seed set	% Germination
1. E. roseum x Lamyi 621 Skåne x 15	4	55	78
2. E. Lamyi x roseum 15 x 621 Skåne	9	55	85
3. E. roseum x obscurum 621 Skåne x Pn 763	5	55	74
4. Reciprocal	6	60	70
5. E. roseum x brevipes 621 Skåne x Birmingham	11	44	85
6. E. roseum x nerterioides 621 Skåne x Scot.	8	Cross failed	-
7. Reciprocal	5	"	-
8. E. Lamyi x adnatum 15 x Pn 661	14	50	45 ?
9. E. Lamyi 15 x tetragonum PS163	7	25 ?	83
10. E. Lamyi 15 x obscurum Pn 763	6	70	90
11. Reciprocal	3	58	75
12. E. Lamyi 15 x palustre 619	10	50	85
13. Reciprocal	6	45	75
14. E. Lamyi 15 x alsinifolium R1621	7	50	80
15. Reciprocal	5	40 ?	65
16. E. obscurum Pn763 x palustre 618	3	50	Germination not tested
17. Reciprocal	5	45	"
18. E. obscurum x alsinifolium Pn763 x R1621	5	35 ?	"
19. Reciprocal	4	40	"
20. E. obscurum Pn763 x tetragonum PS163	4	40	65
21. E. obscurum x adenocaulon Pn763 x SL	5	34 ?	70
22. Reciprocal	3	30 ?	55
23. E. adenocaulon SL x Lamyi 15	6	35 ?	Not tested
24. E. adenocaulon SL x palustre 619	6	50	"
25. E. adenocaulon x alsinifolium SL x R1621	7	45	70
26. Reciprocal	3	45	65
27. E. adenocaulon x roseum SL x 621 Skåne	4	25 ?	Not tested
28. Reciprocal	5	30 ?	"
29. E. alsinifolium x roseum R1621 x 621 Skåne	4	60	78
30. E. alsinifolium x palustre R1621 x 619	3	60	68-70
31. Reciprocal	5	75	75

? alone indicates low percentage ; with bracket indicates reciprocal differences.

did not succeed in either direction. Good seeds are not conspicuously concentrated in a particular portion of the capsule; but where seed set is below 50% the upper half of the capsule appears to contain most of the perfect seeds.

Undeveloped seeds are variable in size. Empty ones are very minute, while those containing imperfect embryos which failed to develop at one stage or the other range in size from minute to nearly approaching a well-developed seed. The proportion of such seeds varies from cross to cross. Where the percentage of seed set is higher than 60%, the number of these intermediate seeds is low; but it is higher if the seed set is below 50%.

Detailed investigation of seed development has been made in the following crosses:

1. & 2. <u>E.roseum x nerterioides</u> and the reciprocal:

Only empty seed is obtained.

<u>Embryo</u>: In both the crosses, it appears that the fertilization is not affected. Ovules fixed after 2-4 days of pollination release embryo sacs without dividing embryos. After 6-8 days the embryo sacs disintegrate entirely and ovules become empty.

<u>Endosperm</u>: The endosperm disintegrates with the embryo sac.

3. <u>E.obscurum x adenocaulon</u>:

Well-developed seeds are usually confined to the upper part of the capsule. Aborted seeds are shrunken, either empty or containing minute dried embryos of various size.

Embryo: Development commences with regular division in the zygote and the pro-embryo, resulting in normal differentiation of the octant embryo after 3 days of pollination. After this, some of the embryos get retarded in their growth and degenerate before reaching the cordate stage. This is revealed in preparations of capsules fixed 8 days after pollination in which one finds only cordate embryos in developing ovules, side by side with empty ovules which have long ceased to grow. A few of the cordate embryos seem to possess bigger suspensors (Fig. 10c) than usual. But no other irregularity in development has been observed. Some sort of selection is exercised at this stage; some of the embryos grow to maturity, while others fail at different stages of their growth.

Endosperm: Free nuclear division in the endosperm is quite normal. Its disintegration occurs only after the cordate stage; but in cases, where embryo fails to reach cordate stage and degenerates early, the endosperm too disintegrates quickly.

4. E. adenocaulon x obscurum:

Embryo: As in the reciprocal, the early divisions are regular. It should be pointed out here that the embryo sac is very often 5-nucleate in E. adenocaulon (Fig. 10d). It is not known how this extra nucleus is derived and what is its function. The first stage of failure ensues somewhere after the octant stage. Failing embryos never reach the cordate stage and degenerate quickly. However, a majority of the embryos attain the cordate stage. A characteristic suspensor (Fig. 10e) of 4 cells laid down one over the other is seen with all the cordate embryos. Now a second phase of failure begins. Some of these embryos fail to develop to maturity. The rest proceed normally to develop into mature seeds.

Endosperm: There is no anomaly of any kind in endosperm development.

5. & 6. E.palustre x alsinifolium and the reciprocal:

Seed set is very high. A mature capsule contains fully developed seeds with perfect embryos with a few empty seeds. The empty seeds are usually contained in the lowermost part of the capsule. There are no seeds of intermediate size containing imperfect embryos.

Embryo: A few ovules in the extreme lower part of the capsule are probably not fertilized because no dividing embryo has been found in them in any preparation. All fertilized ovules develop with equal rate throughout the capsule. However, a few of the octant embryos seem to be too weak to continue growth and die very soon. Ovules from any one capsule older than 6 days after pollination contain embryos of only one stage of growth through-out consistent with the age of the capsule. Ovules which have failed to grow contain nothing and they are found here and there along with the viable ones.

Endosperm: There is no abnormality in endosperm growth.

7. E.Lamyi x obscurum

Capsules are well-developed and seed set is very high. However, the seeds are of different size; 15-20% (varying from capsule to capsule) are completely empty, 30-40% are shrunken seeds which contain imperfect embryos of one stage or the other, and the rest are fully developed seeds. This count is based on 6 capsules. Many of the imperfect seeds approach normal size and have germinated. An arbitrary judgement has been exercised to consider some of these as good seeds and others as bad ones on size.

Embryo: Embryo development is normal. Failure is gradual and begins with retardation of growth in various embryos which cease to grow at different stages. Very young embryos, which fail to grow, degenerate quickly and the ovules become empty.

Endosperm: No irregularity in endosperm growth could be confirmed.

DISCUSSION OF THE RESULTS

The formation of viable seed following cross pollination depends on a long series of developmental processes. In incompatible crosses, the series is disturbed at one stage or another and this results in seed failure. Out of the 117 combinations of interspecific crosses of *Epilobium* reported in the present work, 20 crosses have failed to yield any good seed. The rest have yielded good seeds of varying numbers from cross to cross. The lowest seed set has been found in the cross *E. fleischeri x hirsutum* which produced only 4 seeds out of a total of 5 capsules pollinated. On the other hand, the seed set was as high as 75% in the cross *E.palustre x alsinifolium*. In any case, it appears that the interspecific crosses of *Epilobium* usually result in a reduction of seed set. On one extreme the number of the hybrid seeds may be reduced to zero in some crosses while, on the other, the reduction may be only very slight in other crosses.

The crosses which have failed to set seed include 4 crosses involving *E.nerterioides* with *E.montanum* and *E.roseum* (see Tables 9 and 10) and 16 involving *E.angustifolium* with *E.hirsutum* *E.parviflorum* and *E.montanum* (see Table 7). p.66

In the crosses involving *E.nerterioides*, it has been revealed on the basis of the embryological study that fertilization did not take place. No dividing zygote or pro-embryo was ever seen in any of the preparations. It was observed that there was germination of the pollen grains on the stigma in each of the crosses, but apparently the pollen tubes failed to reach the ovules before the latter aborted. Slowing down of the pollen tube and failure to fertilize the ovules has been observed in many interspecific crosses. In many of the Datura crosses, the failure of the pollen tube to reach the ovules has been recorded (Avery, Satina, and Rietsema, 1959). In the cross D. metaloides x stramonium, the pollen tube either grows too slowly or burst while growing within the style. The cross Gilia splendens x australis usually fails mainly because of the inability of the australis pollen tubes to reach the splendens ovules (Latimer, 1958). Several examples of this sort has been cited by Grant (1963) and it seems that it is one of the very common causes of seed failure in incompatible crosses.

The fact that the slowing down of the pollen tube growth in the interspecific crosses of Epilobium is not uncommon is also shown by the usual confinement of the good seeds to the upper part of the capsule.* In many of the crosses in which the seed set is usually less than 50%, or more particularly which yield only a few good seeds, the capsules are swollen in their upper region with fully developed seeds. It is believed that the pollen tubes are too slow to grow and to reach the ovules in the lowermost part of the capsule. The ovules in the upper

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* See also Appendix 2

part are reached more easily and hence fertilized in time before they abort. However, a second explanation, that this might be due to the shortage of pollen on the stigma during artificial pollination, cannot entirely be ruled out, although every care was exercised to put sufficient pollen on the stigma; and taking all the factors into account, a comparatively slower rate of growth of the pollen tube into a foreign style appears to be the most reasonable explanation of this phenomenon.

It is only in the crosses involving E.nerterioides, as we have observed, that complete failure of fertilization occurs due to the slowing down of the pollen tube growth. In all other crosses, this has only a partial effect. A very few ovules in the lower part of the capsule can escape fertilization, but most of them are fertilized. It is the failure of these fertilized ovules to develop into viable seeds that is more significant and accounts more for the incompatibility in Epilobium crosses.

There is almost no seed set in the crosses between Chamaenerion and Lysimachion (Schizostigma) (see Table 7). The 16 crosses involving E.angustifolium yielded only empty or shrivelled seeds and the 3 crosses involving E.fleischeri did result in a few apparently good looking seeds but these were not viable. Two of them obtained in the cross E.fleischeri x hirsutum were able to germinate, but the seedlings died too young to confirm their hybrid origin. In all these crosses, the seed failure occurs only after fertilization. The main causes are: (a) irregular division in the embryo, (b) slow growth of the embryo, and (c) early dissolution of the endosperm. The irregular division, which takes place quite early, does not only disturb the entire pattern of further growth of the embryo but also disturbs its polarity. The slow growth of the embryo may be due to lack of growth hormones which eventually cause the seed collapse. Most often the irregular division or the slow growth of the embryo commences at the quadrant or the octant stage. These stages of the embryo seem to be very critical. Any disturbance in these stages leads to the seed collapse. In the crosses involving E. fleischeri. however, some of the embryos manage to reach the cordate stage and the slowing down of their growth is apparent only after this stage. It is because of this that the size of the shrunken seeds in the E. fleischeri crosses is often larger. The early disintegration of the endosperm appears to be another factor hastening the seed failure. The endosperm is a nutritive tissue providing nourishment to the young developing embryo. In its absence, the young embryo is likely to be starved to death.

The crosses between members of the Schizostigma result in a moderate amount of seed set; it is very low in the crosses involving E.hirsutum but is fairly high in those involving E. lanceolatum. The main factor contributing to the seed failure in all these crosses appears to be the slow growth of the embryo and its inability to get through the various critical stages of its development and differentiation. In the crosses between E.hirsutum and E.parviflorum, the rate of the growth of some of the embryos slows down soon after the octant stage, while others fail in between the cordate and the torpedo stage. Obviously, all the three stages, the octant, the cordate and the torpedo, are critical for the hybrid embryos of these crosses. The embryos of the cross E.montanum x luteum fail to develop in between the octant and the cordate stage; those of the crosses between E.parviflorum and E.montanum fail mainly at the cordate stage, and those of the E.hirsutum x luteum cross fail at various stages of their development. The seed failure in the crosses between E.hirsutum and E.montanum occurs early; most of the embryos fail to get differentiated into the cordate stage. They all cease development at the 'reversed Trapezium' stage. In E.hirsutum x montanum, the seed failure is initiated slightly earlier than in the reciprocal. Besides, the embryological studies have revealed irregular division of the embryo as another cause adding to the seed failure in E. hirsutum x montanum as in the crosses involving E.angustifolium.

Although the endosperm has been found to be slow in growth and degenerates quite early with the failing embryos of the crosses between *E.hirsutum* and *E.parviflorum* and between *E.hirsutum* and *E.montanum*, no other abnormality has been recorded in other crosses. It is highly doubtful if any abnormality of the endosperm is a positive factor causing seed failure in these crosses.

Fair amount of seed set in the Schizostigma x Synstigma crosses:

The seed set in the crosses involving members of the *Schizostigma* and the *Synstigma* is, on the average, fairly high. In none of the crosses did the embryological studies revealed any irregularity in the division of the embryo. However, the suspensor was found to be slightly bigger than normal in the cross *E.Lamyi x hirsutum* and the synergids were seen to have been dividing in the cross *E.parviflorum x roseum*. How far such abnormalities can contribute to the seed failure is difficult to assess, but it is certain that they can steal food and cause starvation of the young embryos. In any case, the most important factor causing seed failure appears to be the inability of the embryos to get through the critical stages of their differentiation. The rate of the embryo growth is slow and the critical stages of differentiation are invariably the octant and the cordate stages. The endosperm growth is also slow in the failing seeds. This adds to the process of the seed abortion but does not seem to be a major factor in itself.

Very high seed set in the Synstigma x Synstigma crosses:

These crosses are very successful in terms of seed set. The division of the embryo in all the crosses is regular and so is the endosperm growth. Some seeds fail because the embryos cease to grow either at the octant stage or at the cordate stage; but the number of such embryos is not large and it varies from cross to cross. Another reason of the failure may be the intense competition amongst a large number of developing hybrid seeds within a capsule. Weaker embryos are slow to grow and to compete.

Reciprocal differences in Seed-incompatibility:

In none of the interspecific crosses, as we have now been able to observe, did the cross succeed in one direction but fail in the other. On the contrary, the crosses either failed or succeeded no matter in which direction the cross was made. Also, the degree of the success or of the failure in the reciprocal crosses is more or less the same. In a few of the crosses, e.g., between *E.luteum* and *E.parviflorum*, *E.hirsutum* and *E.roseum*, *E.parviflorum* and *E.Lamyi*, *E.montanum* and *E.adenocaulon*, and between *E.Lamyi* and *E.obscurum*, there is a reflection of the reciprocal differences in the percentage of seed set. But this difference is of the order of 10-15% and is not highly significant in the light of the total seed output in the crosses concerned. It may be due merely to some chance factor. On the whole, it can safely be concluded that there is little or no reciprocal difference in the seed incompatibility in the interspecific crosses of Epilobium. On the other hand, reciprocal differences in the seed incompatibility have been reported in the crosses between diploid species of many plant genera, e.g., in Nicotiana (Kostoff, 1943), in Datura (McLean 1946), in Primula (Valentine, 1956), in Gilia (Latimer, 1958), and in many others.

In Epilobium, the embryo and the endosperm are both diploid and hence in the hybrids both are genetically identical. In contrast to this, most of the other Angiosperms have triploid endosperm and their reciprocal hybrids consequently possess genetically different endosperm. The fact that the reciprocal crosses of Epilobium happen to possess identical endosperm might have the effect of diminishing reciprocal differences in the seed development.

Conclusions:

The failure of seed set in the crosses involving E.nerterioides, which is a native of New Zealand, indicates that this species is completely isolated as a breeding unit from the European species.

Likewise, Chamaenerion and Lysimachion are quite separate breeding groups.

Within Lysimachion, E.hirsutum is marked off from the rest by rather low crossability.

Uniformly high seed set and less of irregularity in the seed development in the Synstigma x Synstigma crosses indicate that there is possibly less variation in seed development in crosses between inbreeding species than in crosses in which outbreeding species are involved.

Germination of the hybrid seeds on the whole is uniformly high.

GROWTH, VIGOUR AND FERTLITY OF THE HYBRIDS

General:

The characteristic features of *Epilobium* hybrids, on the basis of previous information, have already been outlined in our survey. These mainly relate to the vegetative and reproductive anomalies of various kinds in the hybrids. At the same time, the hybrids very often differ reciprocally in expression of these characteristics.

The hybrids raised for the present study are listed in Table 11. The germination of the hybrid seeds and the transplantation of the seedlings into pots or into boxes followed the same course as that described for the species.

Lethal or non-viable hybrids died at various stages of their growth. Some died soon after their germination while still in the petri-dishes. Others died at different periods after their transplantation. It is possible that the death of a few seedlings might have occurred due to physical injury received during handling.

When there was a large number of seedlings of hybrid available, some of them were grown in the greenhouse in the boxes as mentioned above, while others were grown in a bed in the open. Thus in many cases, two parallel sets of the same hybrid were grown in two different conditions. Hybrids grown in the open

TABLE II

Performance of hybrids on interspecific crosses in Epilobium

♀ \ ♂		angustifolium	fleischeri	luteum U261	hirsutum			parvi-florum		montenum		lanceolatum AF#604	roseum Skäne 621	adenocaulon SL	adnatum		Lamyi 15	obscurum Pn763	palustre 618	alsinifolium R1621	brevipes
					672	NC1	KR5862	SP6262	163	SL621	616				Pn661	tetragonum PS163					
angustifolium			--																o		
fleischeri		-					--														
luteum U261					+		+	+		+											
hirsutum	672			-+							-										
	NC1			-+					+												
	KR5862			-+				+		-			+						-		
parvi-florum	SP6262			+			+			+			+(-)	++		-+	+-	+	+		
	I63					+															
mont-anum	SL621			+			-	+					-				--	++		+	
	616				-																
lanceolatum AF#604				-+				++		-+							-+				
roseum Skäne 621							+	+		-+							++				+-
adnatum Pn661																	++				
Lamyi 15							+	+		-+			++		++	++		--	++	+(-)	+(-)
obscurum Pn763								+		++							--				
palustre 618, 619								+									++				
adenocaulon SL								++													
alsinifolium R1621										+											

--	Lethal
-	Highly disturbed
-+	More or less disturbed
+-	Slightly disturbed
+(-)	Nearly normal
+	Normal
++	Vigorous

were usually less tall but otherwise similar to their respective sets in the greenhouse. Any difference found between the two sets has been noted in the description of the hybrids.

Many of the surviving hybrids have been found to be physically disabled. They are stunted in growth and have usually mottled and curly leaves. Such hybrids invariably fail to flower. Still a large number of the hybrids manage to grow well and produce flowers, but have a low fertility.

The estimate of pollen fertlity is based on counts of well-shaped grains stained in aceto-carmine. Pollen in Epilobium (except in Sect. Chamaenerion) is held together in tetrads. Lewis and Moore (1962) attempted to exploit this fact to detect genetic segregation, if any, associated with the pollen abortion by counting good and bad grains tetradwise, but without much success. I have repeated this exercise in some of the hybrids and the information has been incorporated when found useful. It must be mentioned that the percentage of good and bad pollen varies a good deal from anther to anther and from flower to flower. Weather, especially temperature, appears to have a great influence on this variation.

Assessment of seed-fertility only on the basis of seed set on selfing does not seem to be complete in itself. In a situation of hybridity where pollen is highly sterile, or at least not very fertile, full potentiality of the capsule is not likely to be realised upon self-pollination. Therefore backcrosses were also made and information of seed set under both the circumstances has been given.

CHAMAENERION X CHAMAENERION

1. E.angustifolium x fleischeri: Three extremely weak seedlings were obtained. They died soon after their germination in the petri-dish.

The Reciprocal: Four hybrid seedlings were obtained. One of them died quickly within two days after its germination. The remaining three were transferred to a pot.

Two of these grew very poorly and remained dwarf measuring 18 cm. and 22 cm. in height respectively. After about two months of an unhappy existence, they died of chlorosis. The third one was much taller, 41 cm. high, and survived (Fig. 11). It had healthy leaves, but they began to curl and dry much before the end of the summer. Many lateral branches were given out mainly at the base. Stem slender; leaves narrow as in E.fleischeri but longer, shorter than those of E.angustifolium, and the leaf-margin intermediate in serration.

Fertility: The plant did not flower in 1964.

The rootstock still survives.

CHAMAENERION X LYSIMACHION (SCHIZOSTIGMA)

E.fleischeri x hirsutum: This was the only cross in this

Fig. 11. F_1 hybrid E. fleischeri x angustifolium. Note the curling of the leaves.

section yielding some seeds which germinated. The two seedlings, which came out, were short-lived. They survived for 21 and 25 days respectively and hardly exceeded 5.5 cm. in height with minute yellowish-green leaves. Straight hairs were sparsely distributed all over the stem surface and along the veins of the leaves.

LYSIMACHION X LYSIMACHION

A. Schizostigma x Schizostigma

1. E.hirsutum x luteum: Hybrids between E.hirsutum and E. luteum made by Michaelis are well-known in connection with the cytoplasmic inheritance in the genus. The present hybrid agrees in several details with that described by him (Michaelis 1954, p. 296), especially in so far as the disturbance in the development and the male sterility are concerned. There were three crosses made using three strains of E.hirsutum as female and each of the crosses resulted in completely male sterile F1 individuals. Their anthers were green (but not necessarily reduced to a minute scale), abortive and produced no pollen. The developmental disturbances consisted in early death of many of the hybrid plants at seedling stage and in stunted growth of several others.

The three crosses made were: (i) 672 x U261, (ii) NC1 x U261, and (iii) KR5862 x U261. The F1 hybrids resulting from these crosses had several characters in common. They were all beset with long slender hairs as in E.hirsutum, but rather thinly. The colour of the flower in all of them was crimson or dark red. In other words, the yellow colour of the luteum flower was suppressed in the hybrids. Long runners, a characteristic of E.hirsutum, were also suppressed. And above all, they possessed degenerated anthers.

Nevertheless, they differed in other vegetative characters, e.g. in general height, width of leaves and hairiness. E. hirsutum NCl was more hairy than the other two strains and also its leaves were ± lanceolate. The result was that the NCl x U261 hybrid came out to be more hairy and with comparatively narrower leaves. A comparison of their characters has been given in Table 12, along with thos of the reciprocal hybrid. Because the general habit and the foliar characters seem to be under the maternal influence, the pattern of the vegetative disturbances also differs in the three hybrids. The most conspicuous is the segregation of the 672 x U261 hybrids into tall and dwarf individuals without any intermediates between them. (Fig. 12).

The reciprocal, E.luteum x hirsutum, is normal in growth and also its anthers produce some good pollen, although in a low quantity. The leaves of this hybrid are wider, a character

TABLE 12

A Comparison of Hybrids between E. hirsutum and E. luteum

Characters	E. hirsutum x luteum					E. luteum x hirsutum
	(i) 672 x U261		(ii) KC1 x U261		(iii) KR5862 x U261	
	Dwarf	Tall	Dwarf	Tall		
Height	19-22 cm.	58-75 cm.	7-8 cm.	35-50 cm.	15-36 cm., but a majority of them 19-22 cm.	25-43 cm.
Hairs	Bushy and Pyramidal	Pyramidal, lateral branches near base	Bushy			
Leaves	Petiolate, 2.5 x 0.9 cm. petiole ca. 1.5 cm. long, margin minutely dentate.	± sessile, 8.5 x 2.9 cm. minutely dentate.	minute	± sessile, 6.5-9 x 2-2.6 cm.	Lower leaves distinctly petiolate, upper ones ± sessile and smaller, 8 x 2.6 cm.	Lower leaves ± petiolate, upper ones sessile 4.2 - 5.3 x 1.9 - 2.2 cm. minutely dentate
Flowers	Late flowers, crimson, bud slender and conical	Flower-buds early, buds slender and conical	None	Buds slender and conical	A few flowers, buds slender and conical	Flowers crimson, buds shorter
Sepals	Sepals ± equal to petals.	Sepals lanceolate, yellow-green 0.8-1.2 cm. long	"	Sepals lanceolate 0.8-1.0 cm. long	Sepals 0.8-1.0 cm. long lanceolate	Sepals lanceolate 0.8-0.9 cm. yellow-green
Petals	Petals about 0.5 cm. long, rarely petals exceeding petal.	Petals 1.6-2.0 cm. long and 1.4 cm. broad, notched and clawed	"	Petals 1.5-1.7 cm. long, crimson	1.6-1.8 cm. long, crimson	Petals 1.5-1.8 cm. long, crimson, deeply notched, clawed
Anthers	Minute, green	Minute, green, abortive	"	Minute, abortive	Minute, abortive	Anthers normal, yellow
Pollen	None	Disorganized	"	None	None	Only a few good pollen 10-15%
Stigma	Protruding out of flowers 1.1 cm. long style.	Style 1.8-2.0 cm. long, protruding or not	"	1.6-1.8 cm. long (style)	1.6-1.8 cm, protruding out of the flower	Stigma not protruding 1.5-1.7 cm. long
Seed (i) Self	None on selfing A few following back-crosses.	None 30-35 seeds per capsule on back-crosses	" "	None A few seeds on back-crosses	None A few seeds on back-crosses	None A few good seeds on back-crosses

Fig. 12. F_1 hybrid E. hirsutum 672 × luteum U261; (a) tall and (b) dwarf individuals.

definitely of E.luteum, the female parent. Apart from these differences, the reciprocal hybrids were similar in having long slender hairs and dark red flowers and in the absence of long runners.

The hybrid E.luteum x hirsutum was obtained from two crosses using two strains of E.hirsutum as the pollen parent, namely, (i) U261 x 672, and (ii) U261 x Kr5862. But no noticeable difference was observed between them.

2. E.luteum x parviflorum and the Reciprocal:

Morphology: Both the hybrids are normal in vigour (cf. Geith, 1924, who found E.parviflorum x luteum to be of reduced vigour and the reciprocal to be very vigorous). A comparison, see Table 13, of the characters of the two hybrids shows three main differences, apart from the difference in the general height, between the reciprocal hybrids:

(i) The hybrid E.luteum x parviflorum has shorter but broader leaves.

(ii) The petal size is slightly reduced in E.parviflorum x luteum.

(iii) The stigma protrudes out of the flower in E.parviflorum x luteum.

Fertility: Pollen in both the hybrids is nearly 40-50% good. However, the anthers of the lower row in E.parviflorum x

TABLE 13

Comparison between *E.luteum x parviflorum* and the Reciprocal.

Characters	E.luteum x parviflorum	E.parviflorum x luteum
Development	Normal	Normal
Habit	Erect, well-branched	Erect, profusely branched
Height	35 - 50 cm.	70 - 85 cm.
Leaves	Yellowish green, lower ones slightly petiolate, margin minutely dentate, 5 - 7.5 cm. long and 2.2 - 3.5 cm broad.	Yellowish green, lower leaves prominently toothed, ± sessile, 8 - 11 cm. long and 2.5 - 4.5 cm. broad, smaller above.
Flowers	Pink, fewer	Pink, many
Sepals	Lanceolate, 0.8 - 1.0 cm.	Lanceolate, 0.7 - 0.9 cm. long
Petals	1.2 - 1.5 cm. long, notched	0.9 - 1.3 cm. long, deeply notched
Anthers	Normal, fertile	Normal, fertile, occasionally one or two of the lower row abortive.
Style & Stigma	At level with longer stamens.	Stigma protruding out of flower.

luteum are usually less fertile and produce only 10-15% good pollen. Very often, one or two of them abort and produce no pollen at all. Bad grains are shrunken and are faintly stained in aceto-carmine. Sometimes, micro-pollen grains are also seen in the tetrads (Fig.13a) showing irregularity in meiosis.

Both are self-sterile but set about 25-35 seeds per capsule following backcrosses with either parent.

3. E.luteum x montanum and the Reciprocal.

(see also Geith, 1924; Michaelis & Wertz, 1935).

In brief, our result compares with the previos ones as shown in Table 14.

The reciprocal differences between the hybrids are usually reflected quantitatively, rather than qualitatively. The following are the differences between the reciprocal hybrids:

On the whole, the leaves of E.luteum x montanum are broader and look much more like those of E.luteum. One important qualitative difference, however, was observed in the growth of the hybrids. Nearly one third of the seedlings of E.montanum x luteum showed some abnormalities in having their main axis split into two at an early stage and the normal growth was delayed. After some time, one or two of the lateral branches resumed the growth.

Short glandular hairs are very thinly distributed all over

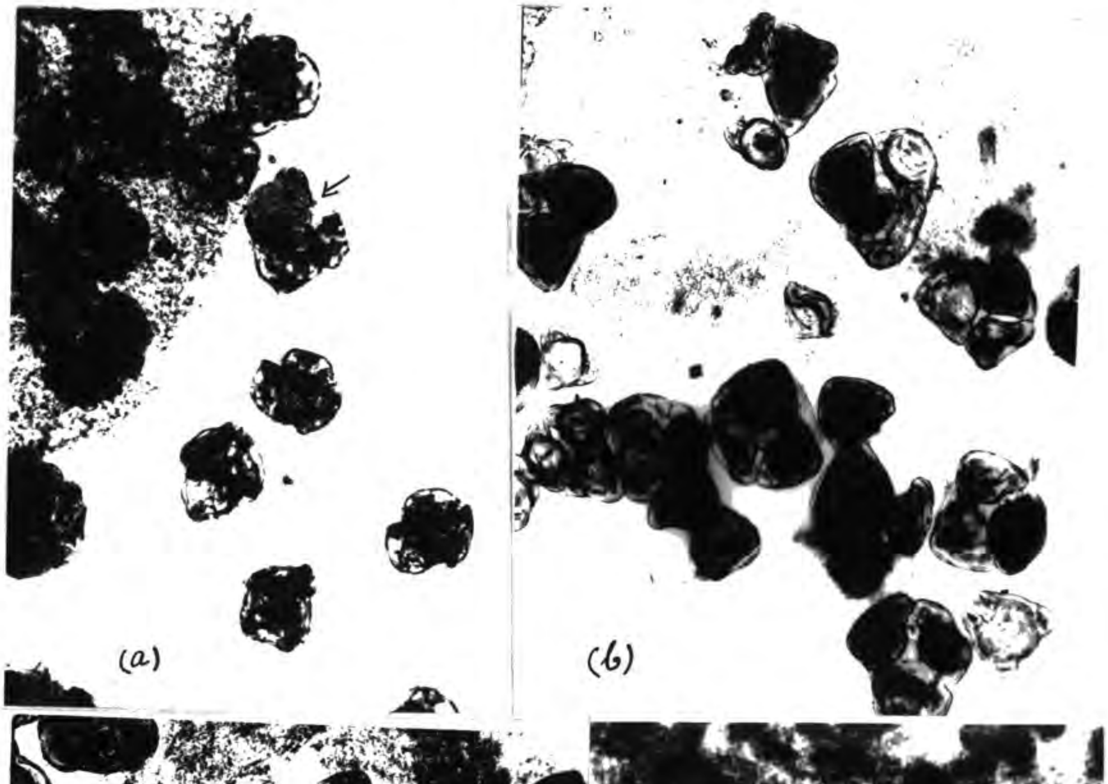


Fig. 13 a-d. Photographs of pollen grains of some hybrids. x c. 150. a- E.parviflorum x luteum ; b- E.hirsutum x parviflorum ; c- E.lanceolatum x parviflorum ; d- E.Lamyi x brevipes. Arrow points to micro-pollen grains.

TABLE 14

Comparison of Results of crosses between E.luteum and E.montanum.

Author	E.luteum x montanum	Pollen	Seed	Reciprocal	Pollen	Seed	Remarks
Geith	Normal vigour	No information		Lethal	-	-	
Michaelis & Wertz	Normal	Fertile	Fertile	Normal	Fertile	Fertile	+
Present Work	"	"	"	"	"	"	++

\+ Reciprocally similar. ++ Reciprocally different in morphology.

TABLE 15

Comparison between the Reciprocal Hybrids of E.luteum and E.montanum

Differences	E.luteum x montanum	E.montanum x luteum
Height	22 - 35 cm.	A few smaller, 27 - 44 cm high, others as tall as 75 cm. but no abrupt distinction between tall and dwarf plants as in E.hirsutum x luteum.
Leaf	3 - 5.5 cm. long and 1.5 - 3 cm. wide.	8.5 - 12.5 cm. long and 3.5 - 4.5 cm. wide.
Petal size	1.7 - 1.9 cm. long	1.0 - 1.2 cm. long.
Sepal	1.2 - 1.3 cm. long	0.5 - 0.7 cm. long
Capsule	2.5 - 3 cm. long	4.0 - 4.5 cm. long

the stem surface and along veins of leaves of both the hybrids. The colour of the flower is pink in both.

Fertility: Both are pollen fertile, producing about 50 - 60% good pollen in the anthers of the longer stamens, but only about 20 - 30% good pollen in the shorter stamens. Capsule contains no good seed, but when backcrossed with either parent, a few good seeds are produced: 10 - 15 seeds per capsule in *E. luteum x montanum* and 15 - 20 in *E.montanum x luteum*.

In the stock of *E.luteum*, obtained from Birmingham, two plants turned out to be natural hybrids (Fig. 14) between *E. luteum* and *montanum*, evidently *E.luteum* being the female parent. These two plants were conspicuous in the whole lot of E.luteum by somewhat smaller and pink flowers. The style was slender and the stigma-lobes were much spreading. In almost all characters, they resembled the artificial F1 hybrid, *E.luteum x montanum*. However, a big difference was found in the pollen fertility. The anthers in this natural hybrid were partially sterile, producing a low amount, about 20 - 25%, of good pollen and the shorter stamens were very often abortive.

4. *E.lanceolatum x luteum*:

(The reciprocal of this hybrid was not raised.)

The development of the F1 hybrid is very much disturbed. 8 seedlings died soon after their transplantation into boxes; 5

Fig. 14. A natural hybrid *E. luteum* x *montanum* growing amidst E. luteum (Birmingham) stock.

others remained alive but were very stunted, about 2.5 cm. in height and fasciated (Fig. 15a); and 9 of them had very slow growth in the beginning but recovered their vigour by sending out healthier lateral branches, still remaining dwarf, about 18 - 25 cm. in height, and decumbent. The rest of the 10 seedlings were much taller, about 40 - 60 cm. high, had normal growth, except that they were very slender and weak and had much longer internodes than either parent. The hybrids grown in the open are more decumbent.

Leaves are narrow, lanceolate, tapering down into distinct petioles, about 4.5 - 7 cm. long and 1.2 - 2 cm. broad, minute and sometimes irregular on dwarf plants.

Flowering vigour of the hybrid is reduced. About one-third of the flowers, very often more than half in the dwarf plants, are abortive. In these flowers, the petal is very much reduced, hardly exceeding the sepal. Such flowers never open fully (Fig. 15b) and fall off at the slightest touch. Normal flowers have 0.6 - 1.2 cm. (highly variable) long petals and 0.5 - 0.7 cm. long sepals. The petals faintly yellow when young but gradually turn pink when old. Longer stamens sometimes exceed petals but are usually at level with the stigma. Pollen is nearly 50% good in the longer stamens, but the shorter ones are more often abortive. Some micro-pollen grains are seen in the tetrads.

Fig. 15. F_1 hybrid *E. lanceolatum* x *luteum*; (a) comparatively healthier plant, and (b) stunted one and it seldom flowers.

A few capsules seem to contain 10 - 15 seeds each on self-pollination. On back-crosses with E.luteum (pollen from E. lanceolatum was not available at the moment) the seed set was about 20 - 25 per capsule.

5. E.hirsutum x parviflorum and the Reciprocal:

(see also Compton, 1913a; Geith, 1924; and Michaelis, 1943, 1954).

Four crosses were made using two strains of E.hirsutum and two strains of E.parviflorum as below:

(i) NC1 hirsutum x 163 parviflorum, and (ii) the reciprocal;
(iii) KR5862 hirsutum x SP6262 parviflorum, and (iv) the reciprocal.

The hybrids E.hirsutum x parviflorum, obtained from the two crosses, are similar in morphology, growth and vigour. The NC1 hirsutum x 163 parviflorum hybrid has slightly narrower leaves than the other hybrid. Their reciprocals are also more or less uniform in themselves. In gross appearance, the reciprocal hybrids are intermediate between their parents, but are thickly beset with simple and straight hairs as in E.parviflorum. The short glandular hairs of E.hirsutum are sparsely mixed with them and are seen on the upper part of the plant, usually near the inflorescence. Unlike those of E.hirsutum, the leaves of the hybrids are not amplexicaul, nor prominently toothed, but are otherwise intermediate in shape and size. The leaves appear to crinkle and dry with the approach of the flowering period. This is somewhat delayed in E.parviflorum x hirsutum; so that quite late in the season, this hybrid still remains healthier than the reciprocal. None of the hybrid possesses long runners of E.hirsutum. Differences between the reciprocal hybrids are mainly quantitative, see Table 16.

Flowering vigour: The crinkling of the leaves in the hybrids, when about to flower, does not appear to damage their flowering vigour. A large number of flowers are borne by each plant.

Fertility: Both E.hirsutum x parviflorum and the reciprocal possess normal stamens and produce about 25% good pollen. The number of good and bad grains per tetrad was carefully recorded in E.hirsutum x parviflorum from several preparations. Tetrads with 3 bad pollen grains (Fig. 13b) were found to be largest in number:

Good Pollen	Bad Pollen	Number of tetrads
1	3	134
2	2	64
3	1	9
0	4	81

Seeds are scarcely set in the capsules of the hybrids grown in the greenhouse. Those grown in the open were able to set a few seeds, about 5-8 per capsule. This difference could possibly be attributed to cross-pollination. Backcrosses always

TABLE 16

Comparison of reciprocal hybrids between E.hirsutum and E.parviflorum

	E.hirsutum x parviflorum	E.parviflorum x hirsutum
Development	Normal	Slightly more vigorous
Growth	Branched, erect	Branched, more branches near base, somewhat bushy
Height	75 - 85 cm. tall	40 - 63 cm. tall
Leaves	8 - 13 cm. long and 1.8 - 3.2 cm. broad, lanceolate	7 - 11 cm. long and 1.9 - 3.2 cm. wide, ovate-lanceolate.
Floral diameter	About 2 cm.	About 1.6 cm
Petal	1.5 - 1.6 cm. long	1.3 - 1.4 cm. long
Septal	0.8 - 0.9 cm. long	About 0.7 cm. long

result in a fair amount of seed set in the hybrids: about 40% or more in NC1 hirsutum x 163 parviflorum and its reciprocal, about 50 - 55% in KR5862 hirsutum x SP 6262 parviflorum and its reciprocal.

6. E.hirsutum x montanum and the reciprocal:

(cf. Compton, 1913a, 1913b; Åkerman, 1921; Geith, 1924; Michaelis, 1954).

The previous work suggest that the hybrids between E.hirsutum and E.montanum are highly disturbed in growth and are sterile. Geith's (1924) E.montanum x hirsutum, however, turned out to be very vigorous. On the other extreme is the hybrid E. hirsutum x montanum-Jena (Michaelis, 1954) in hirsutum cytoplasm which is not viable.

The present results confirm that the hybrids are much reduced in vigour and often unable to flower. The hybrid E.hirsutum x montanum is very stunted in growth and does not flower. Hybrids of this combination were obtained from two crosses: (i) KR5862 hirsutum x SL 621 montanum and (ii) 672 hirsutum x 616 montanum. The hybrid plants from the first cross never exceeded a height of 6 cm. and practically all the stocks, except one, died in the autumn of 1964. The surviving one has given out a shoot in the summer of 1965 but is not likely to grow with vigour. The second cross yielded better hybrid plants, which, although stunted and unable to flower, were about 18 - 32 cm. tall. They all survived last autumn and healthier shoots have been given out this summer (cf. Compton, 1913a). These look intermediate between the parents. Long stolons of E.hirsutum are absent.

The cross E.montanum SL621 x hirsutum KR5862 gave still better hybrids (cf. Compton, 1913b; Geith, 1924). Out of 26 seedlings, only 8 survived, but one of them grew 84 cm. tall and bore a few flowers. Others remained dwarf measuring between 15 to 28 cm. All had curly and mottled leaves (Fig. 16). This hybrid was less hairy than the reciprocal. Long runners are absent.

Only four flowers opened fully and they were left to set seed, if they could, on selfing. But no seed was set. Pollen was only 10 - 15% good.

7. E.parviflorum x montanum and the reciprocal:

(cf. Haussknecht, 1884; Compton, 1913a; Lehmann, 1919; Geith, 1924; and Schwemmle, 1924).

Haussknecht found the reciprocal hybrids to be identical and Compton's E.montanum x parviflorum hybrid tallied exactly with Haussknecht's description. Lehmann, Geith, and Schwemmle found reciprocal differences between the hybrids; E.parviflorum x montanum was male sterile, whereas the hybrid E.montanum x

Fig. 16. F_1. hybrid *E. montanum* SL621 x *hirsutum* KR5862.
Note the curly leaves.

parviflorum was not only pollen fertile but also more vigorous.

Our results agree with Haussknecht's. There are no noticeable reciprocal differences between the hybrids, except that the leaf in the hybrid E.montanum x parviflorum (also see Compton, 1913a) is broader, about 11 x 3.5 cm., than that in the reciprocal, 10.2 x 2.5 cm. In vigour, height, hairiness, flower-size, and fertility, the hybrids are identical.

The hybrid plants are all uniform, vigorous, profusely branched, and about 80 - 85 cm. tall. The stem surface is covered with mixed slender and short glandular hairs. Long slender hairs are mostly present along veins of the leaves. The leaf margin is prominently toothed, but less than in E.montanum proper. The petals are 0.8 - 0.9 cm. long, the sepals being about 0.5 cm. long. On the whole, the hybrids are perfectly intermediate between their parents in morphology.

Fertility: Both E.parviflorum x montanum and its reciprocal are equally pollen fertile. They produce about 50% good pollen. 4 - 6 (very rarely up to 10) seeds per capsule are set naturally on selfing. Backcrosses result in 45 - 50% seed set in both.

Counts of good and bad grains per tetrad revealed that most of the tetrads contained two good and two bad pollens.

8. E.lanceolatum x parviflorum: (The reciprocal was not

grown).

The F1 hybrid is very vigorous, erect, branched and 70 - 85 cm. tall. (After seedlings died after their transplantation, but perhaps due to rough handling). The foliar morphology is much influenced by the female parent, <u>E.lanceolatum</u>. The lower leaves are distinctly petiolate, broadest in the middle, 8.5 - 9 cm. long and 2.5 - 3 cm. broad. The upper ones are ± sessible, broadest near base, acute, 7.5 x 2 cm., smaller and narrower above. All leaves are prominently toothed. Very few hairs are present on the stem surface as well as on leaves. The petal is twice as long as the sepal which is about 0.6 cm. long.

<u>Fertility</u>: The hybrid produces 70 - 80% good pollen; a few anthers produce as much as 90%. Tetrads with 3 good pollen grains are most frequent (Fig. 13c) 20 - 25 seeds are set per capsule on selfing; whereas on backcrossing with either parent, the seed set is 35 - 40%.

9. <u>E.lanceolatum x montanum:</u>

The growth of the F1 hybrid is very much distrubed. A few seedlings die very early and those which survive have slender and weak stems with minute leaves. Each of the surviving plants gives out one or two healthier off-shoots which eventually bear large leaves and function as main stems (Fig. 17). The height is quite uniform, 29 - 31 cm. A normal leaf is about 6 - 8 cm.

Fig. 17. F_1 hybrid E. lanceolatum x montanum. See abortive flower buds.

long and 2 - 2.7 cm. wide, dark green, and distinctly petiolate. Other leaves are minute and irregular. Most often, the leaves are mottled.

The hybrid does not flower; the flower-buds remain minute and never open (Fig.17).

B. Schizostigma x Synstigma

1. E.hirsutum x roseum and the reciprocal:

The hybrids of the reciprocal crosses between E.hirsutum and E.roseum are known to differ according to the strains used (Michaelis, 1954). Geith (1924) did not succeed in getting a hybrid; whereas Schwemmle (1927) did because of the use of different strains of the species concerned.

Our results are given in Table 17.

Thus it appears that the reciprocal hybrids do not differ in their fertility; but they do so in their vegetative characters, although mainly quantitatively.

2. E.Lamyi x hirsutum:

(The reciprocal was not raised).

The F1 hybrid is normal in vigour, about 58 - 68 cm. tall and profusely branched (Fig. 18). The leaves are narrow, lanceolate, 6.8 - 8.6 cm. long and 2 - 2.5 cm. wide, smaller above, ± sessile, and distantly toothed.

Flowering vigour is also normal; petals 1.3 - 1.6 cm. long

TABLE 17

Comparison of Reciprocal Hybrids between E.hirsutum and E.roseum

	E.hirsutum x roseum	E.roseum x hirsutum
Development	Normal, branched	Normal, branched
Height	61 - 70 cm.	45 - 61 cm., one plant was 34 cm
Leaves	Variable, 12 - 14.5 cm. long and 3.4 - 4.0 cm. broad, widely toothed, lower ones with tapering bases, broadest in the middle, upper ones narrower, not tapering.	More variable shape, 8 - 12 cm. long and 3.4 - 4.5 cm. broad, smaller above, tapering base, closely toothed.
Hairs	Long slender hairs and obliquely curved hairs	Slender hairs and curved hairs mixed
Flower	Pink, 1.2 cm. diameter	Pink, 1 cm. diameter
Sepal	0.75 - 0.8 cm. long	0.6 - 0.8 cm. long
Petal	1.3 - 1.4 cm. long	0.9 - 1.3 cm. long, variable
Pollen	40 - 50% good	40 - 50% good
Seed	3 - 5 seeds per capsule on selfing; 20 - 30 seeds on backcrossing.	3 - 5 seeds per capsule on selfing; 20 - 30 seeds on backcrossing.

Fig. 18· Normal F_1 hybrid E. Lamyi × hirsutum.

and sepals 0.7 - 0.9 cm. long.

Fertility: Anthers of both rows are equally good and produce about 60 - 70% good pollen.

No seed is set automatically by selfing. Upon backcrossing with either parent, 25 - 30% seed set is attained.

3. E.hirsutum x palustre:

(The reciprocal was not raised).

Geith (1924) found this hybrid to be of normal vigour and the reciprocal to be very vigorous. Both were able to produce a small amount of good pollen and also 5 - 8 seeds per capsule.

The present results differ from Geith's. We found that the growth of the hybrid was inhibited from the very beginning. Nearly half of the seedlings died after transplantation. The others had stunted growth, 5 - 15 cm. tall, with curly and mottled leaves. One plant, however, measured 30 cm. high and had curly but large dark green leaves. (Fig. 19). The stem surface was covered with downy hairs.

A few flowers were borne on this plant. They were minute, and only partially opened. Sepals and petals were much reduced and the anthers were abortive. When backcrossed with E.hirsutum, 5 seeds were obtained in one of the capsules, but there was no success with E.palustre.

4. E.parviflorum x roseum and the reciprocal:

Fig. 19. F_1 hybrid *E. hirsutum* × *palustre*. Note the stunted growth of the plants.

4. E.parviflorum x roseum and the reciprocal: (Cont'd).

(Cf. Lehmann, 1918, 1925; Geith, 1924; Schwemmle, 1924; Lehmann and Schwemmle, 1927).

The above workers have shown that strong differences exist between the reciprocal hybrids, especially regarding male fertility. The hybrid E.parviflorum x roseum is invariably male sterile and has reduced vigour and petal size.

A comparison between the reciprocal hybrids raised for the present work has been attempted in Table 18.

Thus our results more or less agree with those of previous workers, at least in so far as the fertility of the hybrids is concerned. The vigour of E.parviflorum x roseum does not seem to be reduced, although the reciprocal is undoubtedly more vigorous.

5. E.parviflorum x adenocaulon and the reciprocal:

Both the hybrids obtained from the reciprocal crosses are vegetatively vigorous and alike in many respects. They are profusely branched, normal green, but turn pinkish when old.

The hybrid E.parviflorum x adenocaulon is about 70 - 85 cm. tall in the greenhouse, but 55 - 60 cm. in the open. Nevertheless, it is more profusely branched when in the open. The hybrid E.adenocaulon x parviflorum, on the other hand, is slightly smaller, 45 - 60 cm. tall, but grows alike in the green-house as well as in the open and is more robust. Both the hybrids are

TABLE 18

Comparison of the Reciprocal Hybrids between E.parviflorum and E.roseum

	E.parviflorum x roseum	E.roseum x parviflorum
Development	Normal, branched	Vigorous, much branched
Height	73 cm.	60 - 75 cm.
Leaves	Lanceolate, slightly petiolate, 12.5 - 13 cm. long and 2.8 - 3 cm. wide, margin prominently toothed.	Ovate, distinctly petiolate, broad, 6.5 - 9.5 cm. long and 2.4 - 3.5 cm. wide, margin minutely toothed.
Hairs	Velvety, obliquely appressed.	Velvety, obliquely appressed
Flowers	Reduced, but several	Normal
Sepals	0.25 - 0.4 cm. long	0.4 - 0.5 cm. long
Petals	0.2 - 0.25 cm. long	About 0.7 cm. long
Anthers	Abortive, no pollen	Normal, 50% good pollen
Seed	Self-sterile, 10 - 15 seeds per capsule on backcrosses with both parents.	A few, 2 - 5, seeds per capsule by selfing, 15 - 20 on backcrossing with both parents.++

++ Geith found this hybrid to be seed sterile.

sparingly covered with hairs.

The size of the leaves in both are ± equal, 10.5 x 2.6 cm. in E.parviflorum x adenocaulon, and somewhat variable in the reciprocal, 8 - 10.5 cm. long and 2.5 - 2.8 cm. broad. Leaves are smaller above in both. The leaf-margin is finely toothed and is hairy. It is rather unusual that there are fewer or almost no hairs along the veins. However, there is one remarkable difference between the reciprocal hybrids and it is that the leaf in E.adenocaulon x parviflorum is distinctly petiolate, whereas the leaf in the reciprocal is ± sessile.

Both the hybrids flower in abundance. The hybrid E.adenocaulon x parviflorum has, on the average, smaller flowers; the floral diameter in this hybrid is 0.65 - 0.75 cm., the sepal 0.4 - 0.5 cm. long, and the petal is 0.8 - 1.0 cm. long. The floral diameter in the reciprocal, on the other hand, is about 1 cm., the sepal is 0.5 - 0.6 cm. long and the petal is 0.9 - 1.0 cm. long.

Fertility: Both produce about 25 - 30% good pollen. Tetrads with 2 good and 2 bad pollens, with 3 bad and 1 good pollen, and with all 4 bad pollens, were almost in equal proportion: Table 19. No seed is set by selfing, but backcrosses yield about 20 - 30% seed in both the hybrids.

TABLE 19

A count of tetrads in E.parviflorum x adenocaulon

Good grains	Bad grains	No. of tetrads.
2	2	70
1	3	91
0	4	98
3	1	21

6. E.parviflorum x tetragonum:

The F1 hybrid is dwarf, bushy, measuring from 25 cm. to 38 cm. in height (Fig. 20). The leaves are small and somewhat curled; a few leaves are normal and healthier, 5.8 x 1.5 cm., bearing in their axils large lateral branches. In general, the leaves are apiculate and broadest near base which tapers down to the stem. The hybrid is hairy as in E.parviflorum.

The anthers are malformed and produce about 15% good pollen (cf. Geith, 1924, who found this hybrid, in fact E.parviflorum x adnatum, to be of normal vigour but pollen sterile). The petal size is also reduced; it is only 0.6 cm. long, ± equal to the sepal, which is 0.5 cm. long.

The hybrid is also seed sterile; each of the 4 capsules pollinated with E.parviflorum were found to contain only 2 - 3

Fig. 20. F_1 hybrid E. parviflorum x tetragonum.

seeds.

The hybrid stock has survived by means of short, compact rosettes (very much resembling bulbils in E.palustre), but the growth this summer is still not normal.

7. E.parviflorum x Lamyi and the reciprocal:

The hybrid E.parviflorum x Lamyi is somewhat of reduced vigour. Although it is taller than the reciprocal, it is slender, weak, and less branched and its leaves start mottling with the approach of the flowering season, dry quickly and fall off. It also bears fewer flowers. The reciprocal hybrids have been compared in Table 20.

The differences in fertility of the reciprocal hybrids are most likely due to disturbances in the growth. The flowers in E.parviflorum x Lamyi are quite often ill-formed and the anthers are abortive. Good anthers in this hybrid sometimes contain as high as 80% good pollen. In fact, the percentage of good pollen falls down due to degenaration of some of the anthers.

The longer stamens in the hybrid E.Lamyi x parviflorum exceed the stigma and the shorter ones touch it, but no germination of pollen on the stigma was observed. Tetrads with 4 bad pollens were quite frequent.

8. E.parviflorum x obscurum and the reciprocal:

Geith (1924) did not succeed in getting a hybrid

TABLE 20

A comparison of the Reciprocal Hybrids between E.parviflorum and E.Lamyi

	E.parviflorum x Lamyi	E.Lamyi x parviflorum
Development	Somewhat disturbed	Normal, profusely branched
Height	64 - 74 cm.	55 - 65 cm.
Leaves	7 - 9 cm. long, 2.8 - 3 cm. wide, soon mottling and falling off	8 - 9.5 cm. long, 2.5 - 3 cm. wide, smaller above, normal, tapering base, comparatively narrower
Hairs	Present	Somewhat less hairy
Flowers	Fewer, variable in size	Normal, 1.4 - 1.6 cm. diameter
Sepal	0.5 - 0.6 cm. long	0.6 - 0.7 cm. long
Petal	0.6 - 1.0 cm. long	1.1 - 1.3 cm. long
Anthers	Some normal, others degenerate	Normal
Pollen	40 - 45% good	75 - 90% good
Seed	Self-sterile; upon backcrosses very few seeds per capsule	Self-sterile; upon backcrosses 30 - 40 seeds per capsule

from the cross E.parviflorum x obscurum, but he did obtain the reciprocal one. This hybrid was pollen sterile, but set about 40% good seed on backcrossing.

Our crosses in both the directions were successful and hybrids of normal vigour were obtained. No remarkable reciprocal differences were found between the hybrids, except that the leaves in E.obscurum x parviflorum tended to be less hairy, more greenish, and slightly petiolate.

The hybrids are fairly tall, about 70 - 76 cm. high, and well branched. The leaves are narrow, 7 - 9 cm. long and 1.2 - 2 cm. broad, minutely toothed and broadest about 1/3 rd above the base. Long hairs of E.parviflorum are seen near base of the leaves and on the young tips. On the stem surface are blunt hairs sparsely distributed. The hybrids are intermediate between the parents.

The flowering vigour is normal in both the hybrids, sepals and petals being about 0.5 cm. and 0.8 cm. long respectively. Pollen fertility is 70 - 75%. The hybrids are able to set 10 - 15 seeds per capsule by selfing and produce 30 - 40% good seed on backcrosses.

9. <u>E.parviflorum x palustre and the reciprocal</u>: (see also Geith, 1924).

The present results agree exactly with Geith's findings in vigour and fertility of the hybrids. The only reciprocal difference Geith found was that the hybrid <u>E.parviflorum x palustre</u> was slightly better in pollen fertility than the reciprocal, although both produced only a few good grains.

Both hybrids are normal in vigour; <u>E.parviflorum x palustre</u> is taller, about 70 cm. high, and well branched. <u>E.palustre x parviflorum</u>, however, appears to be more vigorous this year. Last summer, it was less branched and only about 30 - 40 cm. tall. It has survived by means of bulbils and numerous healthy shoots have come out. Both hybrids are velvety in appearance. The leaves are narrow, 6 - 9 cm. long and 1.2 - 1.8 cm. wide.

The anthers of the shorter stamens are usually abortive. Those of the longer ones are normal. On the whole, the percentage of good pollen is very low - in between 20 - 30%.

In none of the hybrids, is seed set by selfing. Only 15 - 20 seeds per capsule were obtained following backcrosses with both the parents.

10. <u>E.montanum x roseum and the reciprocal</u>:

The present hybrids compare with those raised by Geith (1924) in Table 21.

TABLE 21

A comparison of Results of crosses between E.montanum and E.roseum.

Author	E.montanum x roseum	Pollen	Seed	Reciprocal	Pollen	Seed
Geith	-	Sterile	10%	Normal Vigour	Sterile	10% good (10 - 15 seeds)
Present work	Reduced vigour	Sterile, or a few good grains	5 - 6 seeds	Reduced vigour	A few good grains	5 - 10 seeds

Both the hybrids, E.montanum x roseum and the reciprocal, were found to be of highly reduced vigour in our experiments. They are dwarf, varying between 8 cm. to 20 cm. in height, and more or less unbranched. The hybrid E.montanum x roseum is slightly taller and has green, smaller but good looking, leaves. The leaves in E.roseum x montanum, on the other hand, are abnormal. In the early vegetative stage, the leaves are green and normal and look intermediate between those of the parents. But as soon as the hybrid is about 6 - 7 cm. high, they show chlorosis, mottling and crinkling and the lowermost ones absciss early. The younger leaves at the tips are still green, while the older ones turn whitish and droop down (Fig. 21). The leaves measure about 3.5 - 4.6 cm. in length, 1.2 - 2.0 cm. in breadth, and are distinctly petiolate. Those of E.montanum x roseum are a bit smaller.

Only a few flowers, 3 - 8, are borne on each plant. Not all of them open fully. Anthers of both the hybrids are abortive and produce only 10% good pollen. Most of the anthers of E.montanum x roseum contain no good pollen at all.

Some good-looking flowers were pollinated with the parents. The maximum number of seeds set in one of the capsules of E.roseum x montanum was 10 upon backcross with E.montanum.

11. E.montanum x Lamyi and the reciprocal:

The hybrid E.montanum x Lamyi is non-viable. All

Fig. 21. F1 hybrid E.roseum x montanum. Note the chlorophyll deficiency in the hybrids.

the seedlings died early showing an extremely stunted growth, hardly exceeding 2.5 cm. in height with shorter internodes and minute leaves.

The hybrid E.Lamyi x montanum is viable, although highly disturbed in the development. In the seedling stage, it very vigorous; but the leaves start to curl when the seedling is about 8 cm. in height. Some of the hybrid plants remain dwarf, about 15 - 20 cm. high, with their leaves much crinkled. In others, the crinkling is not so severe, and the plants attain a height of 35 - 36 cm. and give out a few lateral branches and flower (Fig. 22). Although the leaves curl and crinkle, sometimes severely, they do not suffer from chlorosis and they are normal green. The leaves are broad, 3.6 x 2.0 cm., evidently a montanum effect.

The flower is about 1.0 - 1.2 cm. in diameter, the sepal is 0.4 cm. long and the petal 0.7 - 0.8 cm. long.

The anthers are all normal and contain about 50 - 60% good pollen. The hybrid is capable of setting 5 - 8 seeds per capsule by selfing. When backcrossed with either parent, the number of seeds increases up to 20 per capsule.

12. E.montanum x obscurum and the reciprocal:

(See also Geith, 1924).

The present hybrids compare with those raised by Geith as in Table 22.

Fig. 22. F1 hybrid E.Lamyi x montanum. The leaves are curly and mottled.

TABLE 22

A comparison of Results of crosses between E.montanum and E.obscurum.

Author	E.montanum x obscurum	Pollen	Seed	Reciprocal	Pollen	Seed
Geith	-	Good	12% good (12 - 15 seeds)	-	Good	12% good (12 - 15 seeds)
Present work	Vigorous	60 - 75% good	30 - 40% good	Vigorous	75% good	30 - 40% good

There are no remarkable differences between the reciprocal hybrids. In gross appearance, they look alike and are intermediate between their parents in several characters. The leaves of the hybrid E.obscurum x montanum however, tend to be more apiculate, narrower, and ± glabrous. The other difference, sometimes noticed, is shorter petals in E.obscurum x montanum on average, but this is not every conspicuous.

In general, both the hybrids are vigorous, profusely branched, and about 30 - 60 cm. tall. The stem surface is covered with short glandular and crisped hairs. Hairs are also present on veins and margins of leaves. The leaves are dark green, 5 - 8 x 2 - 3.8 cm. in E.montanum x obscurum, and 3 - 7 x 1 - 3 cm. in the reciprocal. The raised lines of the stem of E.obscurum are absent or only very faintly present in the hybrids. The leaf-margin is irregularly but sharply toothed.

The hybrids flower in abundance. The flower buds are intermediate in shape and their position vis-a-vis the stem; they are neither erect as in E.obscurum proper nor drooping as in E. montanum. The flower is deep rose with sepals 0.5 - 0.6 cm. long and acute and petals 0.7 - 0.9 cm. long and deeply notched. A few of the flowers are imperfect and their petals hardly exceed the sepals.

The anthers are normal and highly fertile, producing about

70 - 80% good pollen. The longer stamens always exceed the obscurely 4-lobed stigma. The anthers of the lower stamens, however, appear to be less fertile. They often produce only 50 - 60% good pollen.

The hybrids are also highly seed fertile. On average, each capsule contains 20 - 30 good seeds by selfing. Following backcrosses with both the parents, the seed set approaches as high as 30 - 40%, sometimes even more.

Both *E.montanum and E.obscurum* are very variable in height; so are their hybrids. Numerous seedlings of both the hybrids, *E.montanum x obscurum* and the reciprocal, were transplanted very close to one another into two separate boxes. Thus the hybrids were rather accidently put to intense competition. It was found that they did not grow more than 10 - 12 cm. high, nor did they branch, and the number of flowers per plant was reduced to 2 - 5. Nevertheless, no significant reduction in fertility was observed.

Natural hybrids between *E.montanum and E.obscurum* have been reported to be fairly common in Europe and in Great Britain. Judging from the vigour and fertility of the artificial hybrids synthesized in our experiments, it is not surprising that the frequency of their occurrence in nature is so high (see Table 3).

13. *E.montanum x alsinifolium and the reciprocal*:

Here again, there are no remarkable reciprocal differences between the hybrids. The two hybrids are very vigorous in vegetative growth and are profusely branched. They are about 50 - 65 cm. tall, but the hybrid E.alsinifolium x montanum, on the whole, appears to be comparatively smaller, about 50 - 55 cm. high.

The leaves are broad, oval, 8 - 9.5 x 4.2 - 4.6 cm., distinctly petiolate dark green, and almost glabrous. Short blunt hairs of E.montanum are, however, present on the petiole and at the base of the leaf. The leaf-apex is either blunt or acute. A few lowermost leaves are not toothed; the upper ones are closely toothed.

The flowering vigour is normal. The flower is 1.0 - 1.2 in diameter with sepals 0.5 - 0.6 cm. long and petals 1.2 - 1.4 cm. long.

The anthers are normal and fertile. They produce about 55 - 60% good pollen. Counts of good and bad grains per tetrad in E.montanum x alsinifolium have revealed that tetrads with 3 good pollen grains are largest in number (Table 23).

TABLE 23

A count of Tetrads in the Hybrid E.montanum x alsinifolium.

Good grains	Bad grains	No. of tetrads
4	0	81
0	4	90
3	1	123
2	2	45
1	3	15

The hybrids are self-sterile. No seed was found in 10 of the capsules that were examined. When backcrossed to the parents, 10 - 20 seeds per capsule are set.

The stock of E.alsinifolium, R1621, used in the present work, was raised from seeds of the plants grown in the summer of 1962 in the Science Laboratories garden, Durham. Among the plants grown in 1963, an odd individual, conspicuous by its taller height, erect stature, and different leaves, was marked out. This plant has been identified now as E.alsinifolium x montanum. Backcrosses were made with both E.montanum and E.alsinifolium in 1963 and the backcross progeny grown in 1964 summer. Full information on the behaviour of the backcross hybrids is given elsewhere.

14. E.lanceolatum x Lamyi:

(The reciprocal was not raised).

This hybrid shows a number of peculiar features in its growth and development. The plant is nearly 40 - 45 cm. tall, well branched, but very slender with longer internodes and wiry. The lateral branches, which are about 12 - 15 per plant, are thin and their growing tips curve in a tendrillar fashion (Fig. 23). The older leaves which bear the main lateral branches in their axils are larger, 7 - 8 x 1.5 - 2.4 cm., but they soon mottle and fall off. The younger ones on the main stem and

Fig. 23. F1 hybrid *E.lanceolatum x Lamyi*. Note the wiry branches and the abortive flowers.

those on the lateral branches are very small, narrow, 1.2 - 3.6 x 0.25 - 0.5 cm., and green. When the larger leaves have dropped off, the mature plant is seen having only these little leaves. All leaves are acutely apiculate and taper down into distinct petioles.

The flowering vigour of the hybrid is also reduced. Usually 2-3, very rarely 5, flowers are borne on each tip of the several branches. Nearly more than half of the flower-buds never open fully; they are minute and abortive. Normal flowers are pale rose, 0.7 - 0.8 cm. in diameter and with the sepals and the petals measuring 0.5 cm. and 0.8 - 0.9 cm. long respectively.

The longer stamens invariably exceed the obscurely 4-lobed stigma. All 8 anthers of even well-developed flowers are not fully formed; usually one or two of them, most often of the lower row, are abortive. The number of abortive anthers varies from flower to flower.

The highest percentage of good pollen formed in an anther was observed to about 75%; otherwise, the average output of good pollen was 50 - 55%. Tetrads with 2 good grains, those with 3 good grains, and those will all bad grains were found to be more or less equally frequent; although the tetrads with 2 good and 2 bad pollen grains were most often the largest in number.

The hybrid is capable of setting some good seeds by selfing. Although a large number of capsules were empty, a few of them were found to contain some good seed, sometimes as many as 30 seeds per capsule. Backcrosses were not attempted.

C. Synstigma x Synstigma Hybrids.

1. E.roseum x Lamyi and the reciprocal:

The hybrids between E.roseum and E.Lamyi in either direction are very successful. They are normally of good vigour and also highly fertile. A comparison between the reciprocal hybrids in their main characteristic features is given in Table 24.

Thus it appears that the hybrids differ reciprocally both in quantitative as well as qualitative characters. But in fact, the differences are not very sharp and sometimes they are hardly realised without strict measurements and careful observation. Anyway, the fertility in both the hybrids is very high and does not seem to be affected by the direction of the cross.

2. E.roseum x brevipes:

(The reciprocal has not been raised).

This hybrid is very important, because one of the species involved, E.brevipes, is a native of New Zealand.

The F1 hybrid is of normal vigour after it has survived the early stages of its growth. There is a high rate of mortality of

TABLE 24

A comparison of Hybrids between E.roseum and E.Lamyi.

	E.roseum x Lamyi	E.Lamyi x roseum
Development	Vigorous, well branched	Normal, well branched
Height	75 - 84 cm.	42 - 60 cm.
Leaves	Comparatively shorter and broader, 3.5 - 6.5 x 1.6 - 3.2 cm., ovate, sharply toothed, shortly petiolate.	Narrow lanceolate to ovate-lanceolate, 4 - 8 x 1.5 - 3 cm., sparingly toothed, base tapering down into petiole.
Flowers	Many, deep rose	Many, deep rose
Sepals	0.4 - 0.6 cm. long	0.5 - 0.6 cm. long
Petals	0.8 - 0.9 cm. long, deeply notched, veinlets pronounced as in E.roseum.	0.8 - 1.0 cm. long, broader, shallowly notched.
Stamens	Normal, longer ones at level with or exceed stigma, shorter ones cling to it half-way.	Normal, longer ones never exceed stigma.
Pollen	70 - 80% good	70 - 80% good
Seed	40 - 45% good seed by selfing	40 - 50% good seed by selfing

the seedlings; nearly half of the seedlings die early. The hybrid is 18 - 24 cm. tall, bushy in habit and the branches are mainly given out at base (Fig. 24b). Adventitious roots come out at the nodes in the lower region of the semi-prostrate stem. The grown in the open is smaller and ± prostrate. The stem is pink and glabrous. Some of the leaves also redden with the growing age.

The leaves are all very uniform, 2.6 - 3.4 x 1.2 - 1.5 cm., broader than those of _E.brevipes_ but less so as in _E.roseum_, distinctly petiolate, petiole about 0.8 - 1.0 cm. long.

Small flowers are borne singly in the leafy axils, with sepals and petals much reduced and also with abortive anthers. So the hybrid id pollen sterile. However, a few seeds, 3 - 5 per capsule, are set following backcrosses with either parent. The capsules are hairy.

3. _E.adnatum x Lamyi and the reciprocal_:

There are practically no differences between the reciprocal hybrids, except that the hybrid _E.adnatum x Lamyi_ is slightly shorter in height than the reciprocal.

Both the hybrids are quite normal in vigour and are profusely branched. The hybrid _E.adnatum x Lamyi_ is about 40 - 55 cm. tall, erect, and the branches are chiefly at the base. The hybrid _E.Lamyi x adnatum_, on the other hand, is nearly 50 - 70

Fig. 24. Hybrids involving E.brevipes. 24a. E.brevipes ;
24b. F1 E.roseum x brevipes ; 24c. F1 E.Lamyi x brevipes.

cm. high and the lateral branches are more given or less out throughout the whole length of the plant. The stem is covered with whitish crisped hairs and has 4 distinct raised lines.

The leaves in both the hybrids are narrow lanceolate, 3 - 7 x 0.4 - 1.2 cm. with a blunt apex and narrowed down into a short stalk. The leaf margin is sharply toothed.

The hybrids flower in abundance. All flowers are perfect and normal, about 0.9 - 1.1 cm. in diameter, and with the sepals and the petals measuring o.6 cm. long and 0.8 - 0.9 cm. long respectively.

In most of the characters, they appear intermediate between the parents.

Both pollen fertility and seed fertility are very high. About 80 - 90% good pollen is produced by both the hybrids. The capsules of E.adnatum x Lamyi contain 55 - 60% good seed by selfing and those of the reciprocal contain about 60 - 65% good seed.

3a. E.Lamyi x tetragonum:

This hybrid resembles the above two in several characters. The leaves are narrow lanceolate, 3 - 8 x 0.3 - 1.0 cm., and distinctly petiolate. The leaf-margin is more distantly and irregularly toothed than in either E.adnatum x Lamyi or its reciprocal.

The habit of the hybrid, however, differs somewhat in the sense that it is not entirely erect as are the hybrids between E.adnatum and E.Lamyi, rather slightly decumbent at the basal region. This character seems to have been inherited from the pollen parent, E.tetragonum.

The flower is rather large, 1.0 - 1.1 cm. in diameter, with petals measuring 1.0 - 1.2 cm. long. The anthers contain 80 - 90% good pollen and the seed set by selfing is also very high, nearly 60%.

4. E.Lamyi x obscurum and the reciprocal:

The hybrids between E.Lamyi and E.obscurum were found to be non-viable. The seedlings, which were quite healthy when they germinated (cf. a very high percentage of germination), were very slow in growth after their transplantation into pots. They remained alive for a long period, for about two months, but did not exceed a height of 1.2 cm. and bore 6 - 7 pairs of tiny scaly leaves.

5. E.Lamyi x palustre and the reciprocal:

Both the F1 hybrids are very vigorous in growth, and about 30 - 38 cm. tall. There are no remarkable reciprocal differences between them. However, the hybrid E.palustre x Lamyi seems to be slightly more vigorous and more profusely branched than the reciprocal. When the seedlings of E.Lamyi x palustre were transplanted very close to one another, they showed some mottling of leaves and weakening of their vigour in general. When they are transplanted at a good distance apart, they grow normally in good vigour.

The stem is covered with curved hairs all over. The leaves in both the hybrids are ± similar, 5 - 5.5 x 1.2 - 1.6 cm., very uniform in size, broadest near base, sessile, and obscurely or not toothed.

The flowering vigour is slightly reduced in those plants of E.Lamyi x palustre which have mottled leaves, otherwise it is normal. The floral diameter is 0.8 - 1.0 cm., and the sepals and the petals measure 0.4 cm. and 0.6 - 0.9 cm. long respectively. The anthers are normal and contain about 70 - 75% good pollen. But the hybrids are practically self-sterile. Most of the capsule remain empty, although I have observed that the stamens are usually in contact with the stigma. Only very rarely, a few capsules contain 4 - 6 seeds each and it is highly doubtful whether this seed set is due to selfing or due to chance cross-pollination. Following backcrosses with the parents, the seed set is about 20 - 25%.

6. E.Lamyi x alsinifolium:

(The reciprocal was not raised).

The development of this hybrid is somewhat disturbed.

The disturbance is not so visible in the individuals growing in the greenhouse, but those growing in the open are dwarf and obviously less vigorous. The lower leaves show mottling and curl and dry off before the flowering season approaches. The height in general is 27 - 35 cm., and the main stem is semi-procumbent, a habit again inherited from the pollen parent, E.alsinifolium.

The leaves are broad, 6 - 6.2 x 2 - 2.5 cm., shortly petiolate, and with no visible denticulation. They are subglabrous; only a few hairs are present along their veins.

The number of flowers per plant is less than in either parent. The flower-size is variable, and a few of the flowers are abortive. Good-looking flowers are 0.6 - 0.8 cm. in diameter; their sepals measure 0.5 - 0.6 cm. in length and the petals are 0.8-1.0 cm. long.

The anthers are normal and contain 60 - 65% good pollen. Seeds ranging from 2 to 6 per capsule are formed by selfing. Following backcrosses with both parents, each capsule contains 15 - 20 seeds.

7. E.Lamyi x brevipes:

(The reciprocal was not raised).

This is the second hybrid in which the New Zealand species E.brevipes is involved with another of the European members. The hybrid is remarkably intermediate between the two parents. The growth is somewhat disturbed, but, nevertheless, the hybrid grows into a profusely branched bushy plant. The stem is semi-prostrate (prostrate in the open), rooting at nodes in the basal region. The height is 24 - 25 cm. (Fig. 24c).

The leaves are very uniform, intermediate between those of E.Lamyi and E.brevipes, 2.8 - 3.8 x 0.75 - 1.0 cm., with blunt apex and the base tapering down into distinct petiole.

The flowers are borne singly in the leafy axils. The number of flowers per plant is very low. The floral diameter is 0.7 - 0.9 cm., the sepals 0.5 - 0.6 cm. long and the petals 0.65 - 0.8 cm. long. All the 8 anthers are not equally normal; one or two, sometimes all 4 of the lower row, are abortive. Pollen is nearly 50% good; a few micro-pollen grains (Fig. 13d) are seen in the tetrads, suggesting some disturbance in the meiotic division. The capsules are hairy and contain no good seed. Backcrosses with E.Lamyi were attempted and 5 - 6 seeds per capsule were found to have been formed.

STUDIES OF SOME BACKCROSS HYBRIDS

1. The backcross hybrids involving E.montanum and E.obscurum:

A dried specimen of a naturally occurring hybrid between E.montanum and E.obscurum was very kindly sent to me by Mr. P. M. Benoit, under his reference No. 23/756198, collected from Dolgellan, Merioneth, v.c. 48, in 1962. The plant had many ripe capsules with good seeds. This offered me an opportunity for further studies of the hybrid derivatives under cultivation. The specimen in question is now deposited in the herbarium of the University of Durham. From a comparison made, this has been found to resemble the artificial Fl hybrid in most of the details.

40 seeds taken from of of its capsules were sown and 16 seedlings (i.e., 40% germination) were obtained. These segregated into a variety of forms:

16 seedlings

About one-third of the seedlings inhibited.
3 lethal; 2 semilethal.

11 plants, i.e., two-thirds of the seedlings vigorous.

2 plants with entire stigma, tending to be like <u>E. obscurum</u>.

2 plants with swollen stigma, but variable in leaves.

3 plants with obscurely 4-fid stigma, ± like the parent.

4 plants with distinctly but shallowly 4-fid stigma, somewhat like <u>E. montanum</u>.

Morphology of the segregants:

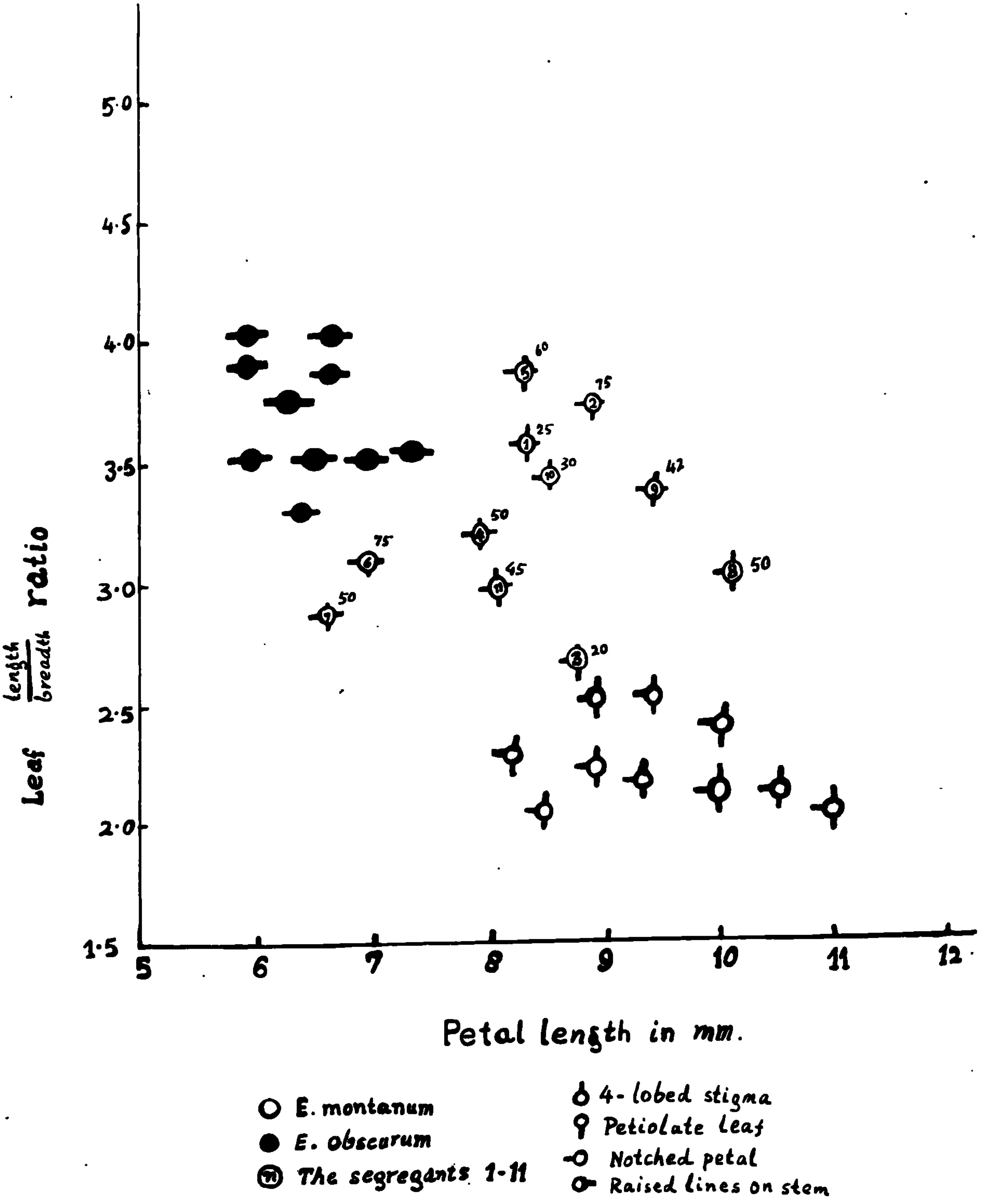
It is quite obvious from the above description that the segregants are extremely variable. Disturbances in the growth, which we frequently observed in the Fl hybrids, are still present in the hybrid derivatives. The inhibited segregants were abnormal in morphology (Fig. 25). Stunted in growth, hardly

Fig. 25. Hybrid derivatives (probably F2) of a natural hybrid between E.montanum and E.obscurum. Note the abnormalities in growth. See text.

raised 0.7 cm. above the ground, the three lethal seedlings had 5 - 6 pairs of leaves spread over in a mosaic fashion. The other two were 14 cm. and 24 cm. tall respectively, but were physically very weak. They too had most of their leaves, which were large and spathulate, confined to their basal part and the upper leaves were minute and irregular. One of them had 3 abortive flowers. The other did not flower.

The remaining 11 segregants were normal and vigorous but differed between themselves in several characters, mainly in the lobing of the stigma, in the petal size and in the length and breadth of their leaves. Some of them resembled E.obscurum to certain extend, some had appearance of E.montanum while still others looked intermediate between the two. To have a full range of variation as exhibited by these eleven hybrid derivatives, they were compared with the parental species and the results illustrated by a scatter diagram (Fig. 26). In the absence of specimens of the parent species from the locality of the original hybrid, E.montanum SL621 and E.obscurum Pn763 were used for the comparison. In fact, these two are also involved in the backcrosses that have subsequently been made.

The comparison is based on the characters which are considered best to differentiate the two species concerned. These are: (1) length and breadth of leaf, (ii) presence or absence of



The number outside the circle represents percentage of pollen fertility

Fig. 26. Scatter diagram of the progeny (F_2) derived from the natural Hybrid E. montanum × obscurum.

petiole, (iii) petal length, (iv) notching of the petal, (v) lobing of the stigma, and (vi) presence or absence of raised lines on the stem. In setting up the basic scatter diagram, the petal length (in mm.) has been plotted along the abscissa and the length/breadth ratio of the leaf along the ordinate. The hollow circles represent E.montanum, the solid ones E.obscurum, and the numbered ones (1 - 11) the segregants. Protrusions of differant lengths in different positions on the circles indiate other characters in different proportions.

As expected, E.montanum falls in the lower right corner of the scatter diagram and E.obscurum in the upper left. The segregants are spread over in between at various position in relation to the parental species. It will be noticed from the diagram that the stigmatic lobing and a lower length/breadth ratio of the leaf do not go hand in hand; the two characters segregate independently. But unless the two characters, 4-lobed stigma and a lower ratio, or inversely the entire stigma and a higher ratio, appear together, the plant is not restored to normality and has reduced fertility. The segregant No. 1 is well within E.obscurum zone but has a more or less 4-lobed stigma. This plant is highly reduced in fertility. Likewise less fertile is the segregant No. 3 which has a swollen stigma (± capitate) but somewhat broader leaves. More fertile individuals usually resemble E.obscurum (Fig.26).

Thus it appears that most of the hybrid derivatives continue to be highly disturbed in vegetative growth or in fertility or in both. Only a few of them, which happen to possess 'a right or compatible combination' of characters, are normal and fertile.

Backcrosses:

The segregants No. 4 and No. 11 (hereinafter referred to as S4 and S11) were backcrossed to the parent species. The stock of E.montanum was SL 621 and that of E.obscurum being Pn763 as mentioned earlier. The selection of S4 and S11 was made because they were intermediate between the two parents, very much like the original hybrid (or F1), and also because they were fertile with no abnormality. S4 and S11 differed between themselves in some vegetative characters; S4 tended to be more like E.obscurum in its leaf and in having raised lines on the stem, while S11 had petiolate and somewhat braoder leaves as in E.montanum.

It was expected that S4 x E.obscurum would produce progeny all like E.obscurum and S11 x E.montanum like E.montanum in vegetative as well as reproductive characters. On the other hand, S4 x E.montanum and S11 x E.obscurum should yield some abnormal progeny.

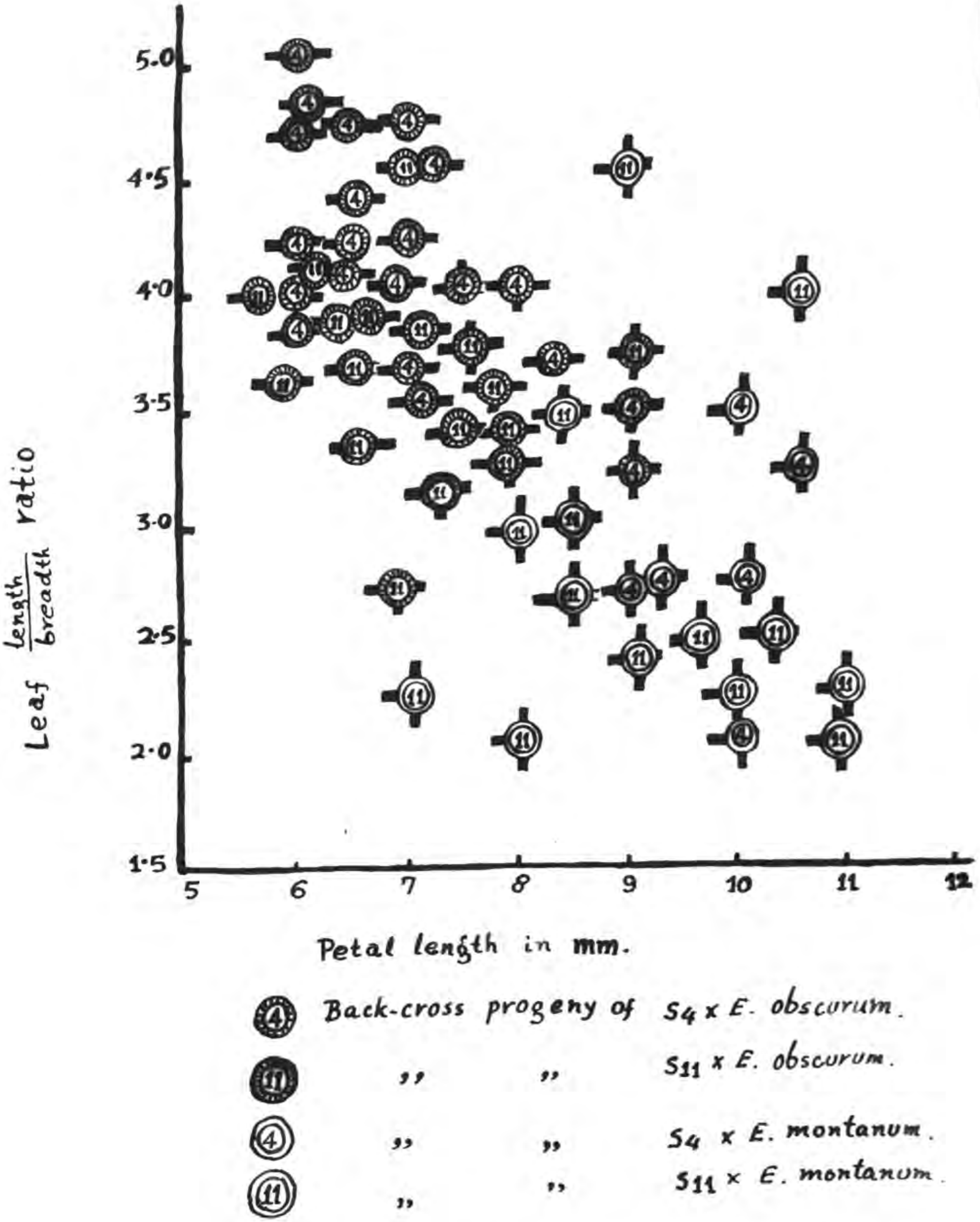
When the backcross progeny from the four crosses were obtained and analyzed again on a scatter diagram (Fig. 27), some interesting points were noted:

(i) The backcross progeny resulting from S4 x E.obscurum and S11 x E.obscurum fall in the upper left corner in heavy concentration. The progeny of S4 x E.obscurum tend to be more narrow-leaved.

(ii) The progeny of S11 x E.montanum are not heavily concentrated at any one point on the graph, rather they are scattered. They are very variable in leaf and petal size. However, their stigma is more or less 4-lobed without any irregularity.

(iii) The progeny of S4 x E.montanum are not so variable as those of S11 x E.montanum, but these still possess raised lines, although faintly, on their stem.

Very few individuals of both S4 x E.montanum and S11 x E. montanum could be analyzed properly as is evident from the diagram. The reason was that most of them were abnormal in morphology. Many of the seedlings of S11 x E.montanum were lethal or dwarf (Fig. 28) in habit, many had irregular leaves and many others had abortive flowers. Likewise, more than half of the backcross progeny of S4 x montanum were highly inhibited in growth. It was not possible to measure or score their abnormal



Other symbols as in Fig. 26.
Fig. 27. Scatter diagram of back-cross progeny.

DURHAM UNIVERSITY HERBARIUM

Name F_2 montanum/obscurum x E^3SL621 E. montanum (1)

Habitat Grown in the Green-house, Science Labs., Durham Univ., Durham.

Locality

Collector V. Thakur,

Altitude

Det.

Date 25. 7. 1964

Fig. 28. Backcross hybrids : a hybrid derivative of E.montanum x obscurum (natural hybrid) backcrossed to E.montanum.

characters.

The contrast between the results of backcrossing with the two species is thus remarkable. The progeny resulting from the backcrosses with E.obscurum re-acquire their original features immediately. The vegetative differences between S4 and S11 seem to be insignificant. On the other hand, the restoration of S4 or S11 into E.montanum appears to be a long story and needs repeated backcrossing for several generations. Here the differences between S4 and S11 also count. S4, which has more of obscurum characters, gives rise to more lethal and abnormal progeny than does S11 when backcrossed to E.montanum.

2. The backcross hybrids involving E.montanum and E.alsinifolium:

As mentioned earlier (page 155), the stock of E.alsinifolium, R1621, contained a hybrid plant, E.alsinifolium x montanum. This was backcrossed to the parents.

The backcross hybrids resulting from both the crosses have been compared together in Table 25.

The backcross hybrids tend to resemble their pollen parents in most of the vegetative characters. But the floral characters and fertility do not appear to have been affected. The fact that they still remain highly seed-sterile indicates that the backcross hybrids are often not better and more useful than the

TABLE 25

A comparison of Backcross Hybrids of *E.alsinifolium* x *montanum*

	E.(alsin. x montanum) x alsinifolium	E.(alsin. x mont.) x montanum
Development	Normal, slender, less branched	Normal, comparatively more branched.
Habit	Erect	Decumbent at base
Height	50 - 55 cm, a few 25 - 30 cm.	30 - 40 cm.
Leaves	Ovate, 4.5 - 7.7 x 2.2 - 3.8 cm., broadest near base, petiolate, margin sharply toothed.	Oval or elliptic, 4.5 - 8 x 2 - 3 cm., broadest in the middle, base tapering down into a short petiole, faintly toothed.
Flowers	Many, large, 0.9 - 1 cm. in diameter.	Comparatively fewer, smaller, 0.8 - 0.9 cm. in diameter.
Sepals	0.5 - 0.6 cm. long	0.5 - 0.6 cm. long
Petals	0.9 - 1.3 cm. long, variable	0.7 - 1.0 cm. long
Anthers	Normal	Normal
Pollen	80 - 90% good	80 - 90% good
Stigma	4-cleft, but the lobes not so spread as in *E.montanum* proper.	Entire, but still irregularly bulged.
Seed	10 - 15 good seeds per capsule	10 - 15 (- 20) good seeds per capsule.

F1 hybrids.

DISCUSSION OF THE RESULTS

The success of a hybrid in breaking down barriers between species depends on two main factors:

(i) The hybrid must be able to compete and to survive; and

(ii) if it survives, it must be capable of reproduction.

Survival of the hybrids:

All the hybrids described in the foregoing pages do not appear to possess an equal amount of fitness for survival. Many are at a great disadvantage; their growth is irregular and highly disturbed and they lack in vigour. The less the vigour, the more precarious are the chances of their survival. Some of the hybrids die very early at the seedling stage, some of them remain dwarf and are abnormal in morphology and many others grow vegetatively very weak.

(i) Of the hybrids raised for the present investigation, the following are lethal and are entirely eliminated sooner or later at an early stage:

E.angustifolium x fleischeri

E.fleischeri x hirsutum

E.montanum x Lamyi

E.Lamyi x obscurum and the reciprocal.

(ii) The following hybrids survive precariously. A great majority of them die at the seedling stage or grow very stunted. Only a few individuals manage to grow to normal height but are physically very weak.

E.fleischeri x angustifolium

E.hirsutum x montanum and the reciprocal (cf. Michaelis's *E.hirsutum x montanum* - Jena - non-viable, 1954).

E.hirsutum x palustre

E.montanum x roseum

(iii) The following hybrids are highly disturbed in their growth. There is no high rate of seedling mortality (although quite a few of the seedlings do die very early), but the development is abnormal. They usually remain dwarf with curly and mottled leaves, or grow unusually longer and slenderer with irregular leaves.

E.hirsutum x luteum

E.lanceolatum x luteum

E.lanceolatum x montanum

E.parviflorum x tetragonum

E.roseum x montanum

E.Lamyi x montanum

E.lanceolatum x Lamyi

(iv) The following hybrids are only slightly disturbed. The disturbances are manifest very late and have little effect on vegetative growth, but nevertheless, the flowering vigour of the hybrids is greatly reduced.

E.parviflorum x roseum

E.parviflorum x Lamyi

E.roseum x brevipes

The remaining hybrids are normal in their growth and development. Some of them rather show heterosis. However, it must be noted that all the hybrids that have been raised were grown under cultivation, perhaps under much protection. What matters really is how they fare in nature in intense competition. The fact that many of the hybrids are relatively smaller when grown in the open suggests that some of them are likely to fare worse in nature. This is supported by our observations on the growth of the hybrids E.montanum x obscurum and the reciprocal. When they were transplanted very close to one another, their height, number of branches and flowering vigour were much reduced. In other words, if the competition is harder, the hybrid is put at a great disadvantage. Differences in growth and vigour of hybrids under different circumstances have also been noted by other workers (Compton, 1913a; Åkerman, 1921).

The hybrids of Epilobium, like their parents, enter into dormanty during the autumn by means of various organs of perennation and send out new shoots early in the next spring. It has been found that the new shoots are sometimes better and more vigorous than the original hybrid stocks (Compton, 1913a). To find out the extent of recovery of the inhibited hybrids, their stocks were carefully looked at in the greenhouse during the autumn of 1964. Some shoots from *E.hirsutum x montanum* (but only of the cross 672 x 616, and not of KR5862 x SL621) stock have certainly come out better in the summer of 1965 and a slight improvement has also been noted in the shoots coming out of the stocks of the dwarf hybrids of *E.hirsutum x luteum*. But others have not recovered. Some of them, e.g. *E.parviflorum x tetragonum*, have rather deteriorated and the stocks of *E.montanum x roseum* and the reciprocal and *E.Lamyi x montanum* have failed to survive.

It is quite evident, therefore, that the inherent disability of the hybrids is not normally got rid of and the highly inhibited hybrids continue to remain so. Perhaps recovery to the normal condition is achieved only when the disturbance is marginal. But this process seems to be too slow to be of much significance.

Fertility:

The hybrids which are not fertile will not be able to give rise to any progeny and will thus be the only generation to exist. Our observations indicate that the *Epilobium* hybrids are greatly reduced in fertility. Only a few of them are fertile to an extent which may result into the production of a number of progeny. The fertility and the productivity of the hybrids, therefore, must be considered in relation to each other. Obviously, the lethal hybrids are automatically eliminated from the race of reproduction. Other causes could be the failure of the hybrids to flower, the abortion of their reproductive organs (anthers and stigma) and their failure to set seed.

(i) Of the hybrids under consideration, the following hybrids, which are highly disturbed in their vegetative growth, are unable to flower:

E.fleischeri x angustifolium
E.hirsutum x montanum
E.lanceolatum x montanum

(ii) The following hybrids flower, but the number of flowers is very much reduced. Most of the flowers are imperfect or aborted.

E.lanceolatum x luteum
E.montanum x hirsutum
E.hirsutum x palustre
E.parviflorum x roseum
E.parviflorum x tetragonum
E.parviflorum x Lamyi
E.montanum x roseum and the reciprocal
E.Lamyi x montanum
E.roseum x brevipes

In fact, almost all the hybrids, which are disturbed and abnormal in their growth, produce less flowers on the whole; and the reduction in the number of flowers is directly proportional to the reduction in their productivity.

(iii) Flowers are produced in the following hybrids, but they are *totally pollen sterile*; their anthers are abortive:

E.hirsutum x luteum
E.hirsutum x palustre
E.parviflorum x roseum
E.montanum x roseum
E.roseum x brevipes

(iv) Most of the anthers are degenerate in the following hybrids; only one or two anthers contain some good pollen and the total output of such good pollen is *very low, about 10 - 20%*:

E.luteum x hirsutum
E.montanum x hirsutum
E.parviflorum x tetragonum
E.roseum x montanum

(v) Of the remaining hybrids, 16 produce below 50% good pollen, 8 below 75%, and only 13 of them (mostly *Synstigma x Synstigma* hybrids) produce good pollen above 75% (see Table 26). Where pollen fertility is below 50%, a few of the anthers, especially of the lower row, appear to be degenerate.

Looking into the seed fertility, we find that the hybrids are highly reduced in seed fertility as well.

(i) Only the following 5 hybrids are able to produce a high amouny (above 40%) of good seed per capsule by selfing:

E.roseum x Lamyi
E.Lamyi x adnatum

TABLE 26

Pollen fertility of the Interspecific Hybrids

Hybrids with 20 - 50% good pollen	Hybrids with 50 - 75% good pollen	Hybrids with above 75% good pollen
luteum x parviflorum +	luteum x montanum +	lanceolatum x parviflorum +
parviflorum x luteum +	Lamyi x hirsutum	parviflorum x obscurum
montanum x luteum +	roseum x parviflorum	obscurum x parviflorum
lanceolatum x luteum +	Lamyi x montanum	montanum x obscurum
hirsutum x parviflorum +	montanum x alsinifolium	obscurum x montanum
parviflorum x hirsutum +	alsinifolium x montanum	roseum x Lamyi ◊
parviflorum x montanum +	lanceolatum x Lamyi	Lamyi x roseum ◊
montanum x parviflorum +	Lamyi x alsinifolium ◊	adnatum x Lamyi ◊
hirsutum x roseum		Lamyi x adnatum ◊
roseum x hirsutum		Lamyi x tetragonum ◊
parviflorum x adenocaulon		Lamyi x palustre ◊
adenocaulon x parviflorum		palustre x Lamyi ◊
parviflorum x Lamyi		Lamyi x parviflorum
parviflorum x palustre		
palustre x parviflorum		
Lamyi x brevipes		

◊ Hybrids involving members of the *Synstigma* group.
\+ Hybrids involving members of the *Schizostigma* group.
The rest involve *Schizostigma x Synstigma*.

E.Lamyi x roseum   E.Lamyi x tetragonum

E.adnatum x Lamyi

These are all Synstigma x Synstigma hybrids.

(ii) The following hybrids produce about 20 - 30 seeds per capsule by selfing:

E.lanceolatum x parviflorum   E.montanum x obscurum

E.lanceolatum x Lamyi   E.obscurum x montanum

(iii) The capsules of the following hybrids contain 10 - 15 seeds each; all the capsules are not equally fertile, some might contain nothing:

E.lanceolatum x luteum

E.parviflorum x obscurum

E.obscurum x parviflorum

Of the remaining hybrids, only 9 of them produce 3 - 8 (very rarely 10) seeds per capsule and the rest (including the 5 pollen sterile ones) are not able to set seed in their capsules by selfing.

Nearly all of them were backcrossed to their respective parents and the percentage of seed set was increased and also there was some seed set in those which were ordinarily self-sterile. It is possible that some of the hybrids, especially those with large flowers, e.g., the hybrids involving E.luteum and E.hirsutum between themselves or with some other species, which are able to attract insects, will set a fair amount of seed upon cross-pollination and will thus be able to produce hybrid progeny. But the occurrence of cross-pollination will certainly be of limited extent and perhaps is not likely to occur in most of the hybrids. Thus the low production of seed by the hybrids sets a limit on their productivity.

CHAPTER V

CYTOLOGY

General

Löve and Löve (1961, pp. 256 - 7) have recently summarised the chromosome counts in *Epilobium* and all the European species have the same number, 2n = 36. The most recent paper of Raven and Moore (1964) on the chromosome numbers of the British Epilobia appeared at a time when I had almost completed my own counts. The number, 2n = 36, is confirmed in all the cases.

The genus, as a whole, does not seem to be a suitable plant material for chromosome preparations. Difficulties in fixation and staining have often been reported (Michaelis, 1954, p. 293; D.M. Moore, oral communication).

Material and Methods

Both mitotic and meiotic chromosome counts of the plants listed on the pages 18 - 22 have been made. The mitosis has been studied in the meristematic root-tip cells and the meiosis in the pollen mother cells. No mitosis was looked into *E. fleischeri*, *E.parviflorum* 675, *E.lanceolatum*, *E.obscurum* and *E.nerterioides*. Similarly time did not permit to examine meiosis in *E.hirsutum* NC1, *E.montanum* PL63 and 617, *E.roseum*, *E.adnatum* and *E.brevipes*.

Several methods of fixation and staining of the root-tips and the anthers, including those described by Lewis, Raven, Venkatesh and Wedberg (1958) and Kurabayashi, Lewis and Raven (1962), were tried. None seemed to work satisfactorily. The following gave slightly better results.

The root-tips were pretreated in para-dichlorobenzene for 3 - 4 hours and fixed overnight in 1:3 acetic alcohol. They were hydrolyzed in N HCl for 30 minutes at 60°C, washed in distilled water, stained in leuco-basic fuchsin, and squashed in a drop of aceto-carmine. The combination of feulgen and carmine improved the staining.

The flower buds were fixed in 1:3 acetic alcohol, hydrolyzed in N HCl for 25 - 30 minutes at 60°C, stained in feulgen, and squashed in a drop of aceto-carmine.

Chromosome Number

In all the counts, the chromosome number 2n = 36 was confirmed. The mitotic chromosomes are very small (Fig. 29a) and no appreciable difference in their size is detected. Each species appears to have more or less morphologically similar chromosomes. There is no irregularity in the meiotic division in any of the species. The 18 bivalents are distinct (Fig. 29b) and they devide very regularly.

Meiosis in the Hybrids

The meiotic division has been studied in the following hybrids and normal pairing of the chromosomes (Fig. 29c) has been observed in each.

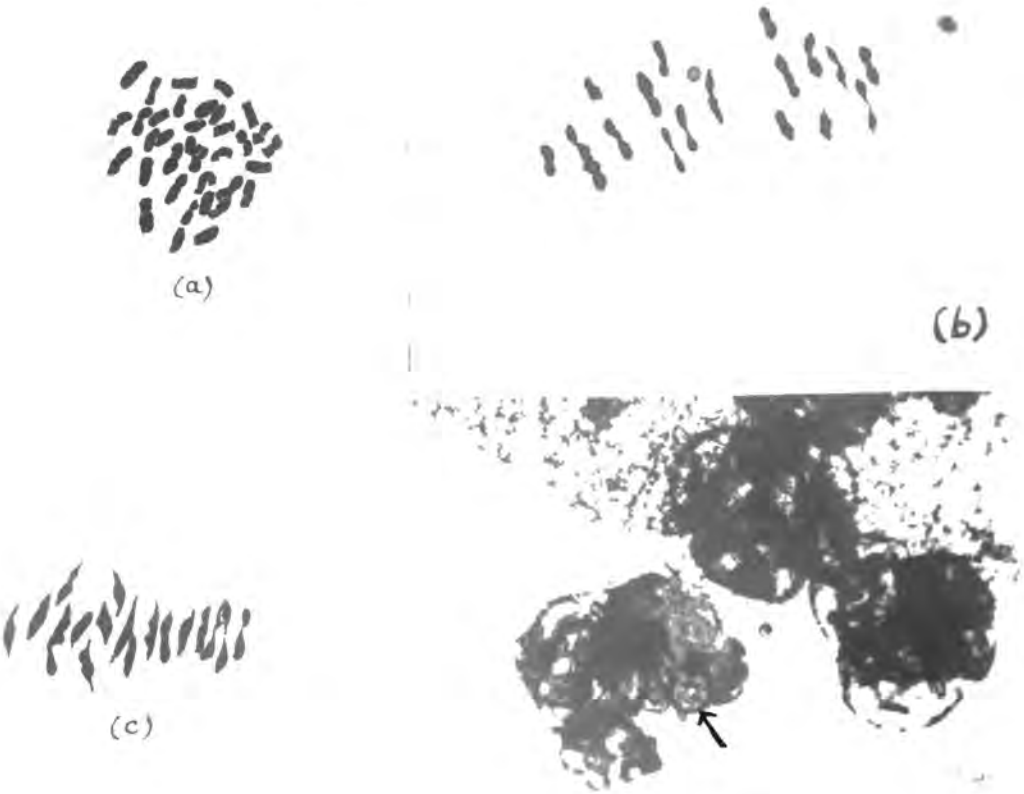


Fig. 29. a. Camera lucida drawing of mitotic chromosomes of E.montanum SL621, x 1800 ; b. photomicrograph of meiotic chromosomes (18 II) of E.parviflorum SP6262, x 1800 ; c. camera lucida drawing of meiotic chromosomes of E.montanum x obscurum (18 II) x 1800 ; and d. photograph of pollen grains in tetrads of E.parviflorum x luteum showing micro-pollen grain with an arrow , x 300.

Hybrids	No. of bivalents at MI
1. E.luteum x hirsutum +	18
2. E.hirsutum x parviflorum	18
3. E.parviflorum x montanum	18
4. E.parviflorum x luteum +	18
5. E.parviflorum x adenocaulon	18
6. E.Lamyi x parviflorum	18
7. E.montanum x obscurum	18
8. E.obscurum x montanum	18
9. E.montanum x alsinifolium	18
10. E.roseum x Lamyi	18
11. E.palustre x Lamyi	18
12. E.Lamyi x alsinifolium	18

In all these hybrids, regular pairing at metaphase I has been observed. In nearly all of the hybrids, anaphase I has also been seen; and in some hybrids, metaphase II has also been seen. In none of these, have any irregularities been seen. As has already been reported, all of these hybrids show some degree of sterility and all produce some shrunken pollen grains. In addition, some hybrids (including those marked with an asterisk) produce micro-pollen grains as well (Fig. 29d); the number of micro-pollen grains per tetrad is variable. In view of the apparent regularity of meiosis, it is difficult to account for the occurrence of these micro-pollen grains; and it is possible that further observations might show that failure of pairing and the production of univalents sometimes occurred.

Discussion

The fact that all the European species of Epilobium possess the same chromosome number (and probably morphologically alike) is very remarkable. This shows that they are, in all probability, a compact and homogeneous group. Michaelis (1954) thought they were an ideal group of plants to investigate the role of cytoplasm in the species differences and he attributed many differences between the species to the cytoplasmic genes. Recently, Kurabayashi, Lewis and Raven (1962) have reported species with $2n = 24$, 26 and 30 and thus it has to be taken that all the species of the genus throughout the world do not have the same number. Straub's (1941) hypothesis on the phylogeny of the genus that the chromosome number $n = 18$ of Epilobium was a result of hybridization in the long past between two species, one with chromosome number $n = 11$ as in Circaea or in Fuchsia and the other with $n = 7$ as in many genera of the Onagraceae is also now seen to be unlikely. The basic chromosome number in the genus should now be regarded as $x = 6$.

Regular meiosis in the interspecific hybrids of Epilobium has been reported by almost all the workers who have been able to investigate meiosis in the hybrids (Håkansson, 1924; Hair, 1942; Lewis and Moore, 1962; and Raven and Moore, 1964).

CHAPTER VI

DISCUSSION AND CONCLUSIONS

Validity of the Results

Our results, on the whole, are not very different from what the earlier workers have found in the interspecific crosses of *Epilobium*. However, there are some disagreements in a few individual crosses which we have already mentioned at appropriate places. Geith (1924) failed to obtain hybrids from his crosses between *E.hirsutum* and *E.roseum* and *E.parviflorum x obscurum*. Also, he obtained a lethal hybrid from the cross *E. luteum x montanum*. We, on the other hand, have been able to get perfectly viable hybrids from these crosses. In fact, Schwemmle (1927) also succeeded in getting hybrids between *E. hirsutum* and *E.roseum.* The statement of Lehmann (1925) that hybrids with *E.parviflorum* as their mother generally tend to be sterile also does not find support with us. The hybrid *E.parviflorum x roseum* is male sterile, but the remaining 9 hybrids with *E.parviflorum* as their female parent are not, although there is some reduction in pollen fertility in a few of them.

The differences of the nature pointed out above may raise doubt as to how reliable are the results obtained in the present work. Truly speaking, almost all the previous workers have obtained results in several crosses differing from each other's in degree of viability and fertility of the hybrids. It is now believed (Michaelis, 1954) that these differences are due to use of different strains by different workers in the same interspecific cross. There are various geographical strains of the same species and when these are crossed to some other species they give different results. (See also our own experiments, Table 11, p105) Thus in assessing the validity of the results of hybridization experiments, this variability has to be borne in mind.

A. Bearing of the Results on Problems of Isolation

The results obtained from the breeding experiments reported in the present work reveal a number of isolating mechanisms operative in the species of Epilobium under our consideration. Usually the various isolating mechanisms seem to be working in combinations to produce effective barriers. No single isolating mechanism, except in a few cases, appear to be all powerful in itself. In fact, a majority of species of the plant or animal kingdoms are separated not by one but by combinations of different isolating mechanisms. We shall give first an account of the main isolating factors working in the genus.

External Isolating Factors

Most of the species of Epilobium described in this account have geographical distributions which overlap considerably, so that geographical isolation is of relatively little importance. Differences in habitat between species exist and often play an important part in isolation, e.g., the alpine and woodland species are often well separated. However, we shall say no more about this aspect of the subject but concentrate on those rather numerous cases in which two or more species occupy similar or adjoining habitats and are in flower at about the same time. Under these circumstances, the pollination mechanism seems to play an important part.

The number of the natural hybrids known, the ease with which several workers have been able to synthesize artificial hybrids, and the high success of the present crosses all confirm that they are readily crossable. The question one asks why is the frequency of occurrence of the natural hybrids so low. The occurrence of the natural hybrids has been examined carefully in a previous section and it was noted that many of the hybrids were rare or infrequent.

Valentine (1951) suggested, with special reference to the distribution of E.hirsutum and E.parviflorum in the British Isles, that self-pollination prevented hybridization between the two species, although they were crossable and lived together in many localities. Lewis and Moore (1926) came forward with the same conclusion with respect to E.adenocaulon and E.brevistylum in America.

The observations on pollination mechanism (Chapter III) have shown that all the members of the Division Synstigma are self-pollinated. The result is a low frequency of the hybrids in nature between them. Referring back to Table 3, we find only one hybrid involving two members of this group in the last column of the most frequent natural hybrids. There are 4 hybrids tabulated in that column. Two of them are between Schizostigma and Synstigma and the fourth involves two members of Schizostigma. Likewise, in the third column of the frequent hybrids in the same Table, only 3, out of 8, are between members of Synstigma and the rest are Schizostigma x Synstigma hybrids. As we shall see later, the species of Synstigma are separated from one another by relatively weaker genetic barriers than are the cross-pollinated species of Schizostigma. In spite of this, the number of natural hybrids between members of Synstigma is no greater than that between members of Schizostigma. It is very interesting that such a balance is maintained in nature between the isolation caused by genetic barriers and that caused by mere self-pollination with no or little genetic barriers. The fact that the self-pollinated species of the genus fail to hybridize is supported by the rarity of natural hybrids in Epilobium in New Zealand. The New Zealand Epilobia all belong to Synstigma and are self-pollinated (Brockie, 1959). However, it must be emphasized that self-pollination does not altogether prevent hybridization; it merely limits the rate of gene-exchange.

Internal Isolating Factors

(i) The growth of pollen tube down into the style is inhibited in the crosses involving E.nerterioides. The pollen tube fails to reach the ovules and thus the fertilization is not affected.

(ii) The inhibition of pollen tube growth is effective in other crosses in reducing seed set. This effect is deduced from the fact that the good seeds are usually confined to upper part of the capsule in those crosses. Obviously some ovules in the lower part of the capsule are not fertilized because the pollen tube does not reach them in time. The number of such unfertilized ovules varies from cross to cross indicating the measure of the effectiveness of this factor. The significance of the slow growth of the pollen tube in these crosses lies not in the total prevention of the fertilization but in limiting its range.

(iii) With the exception of the crosses involving E. nerterioides, fertilization does take place in all other crosses of Epilobium, but all the fertilized ovules do not necessarily develop into mature seeds. Failure of the seed occurs because of failure of the embryo. The embryos fail to pass through the various stages of their development. In the crosses involving E.angustifolium, there occurs a total failure of seed set; while in many other crosses, the seed set is very high. Thus the extent to which seed abortion takes place depends upon the strength of the barrier or barriers working to this end.

(iv) In some crosses, the endosperm has been observed to grow slowly and irregularly and to disintegrate quite early. But this is often accompanied with irregularity and slow growth of the embryo as well and therefore it is difficult to say if the endosperm failure is an independent and primary cause of seed collapse. At any rate, it adds much to the seed failure.

(v) In the crosses E.parviflorum x montanum and E.parviflorum x roseum, the synergids were seen to be actively dividing while the embryo had failed. This type of abnormality is not very common, but it is believed that such abnormal growth of extra-embryonic tissue within the embryo sac is certainly at the cost of the hybrid embryo.

The only information of seed failure in Epilobium crosses with which we can directly compare our results is available in the work of Michaelis (1925) who investigated the development of the hybrid embryos of the crosses between Chamaenerion and Lysimachion. Geith (1924) has reported failure of his crosses between E.hirsutum and E.roseum and of E.parviflorum x obscurum probably because no viable seed was obtained in these crosses, but he did not give details of the failure. Michaelis (1925) made a detailed embryological investigation and found histological abnormalities associated with the seed failure, which we have already mentioned in the introductory section. None of Michaelis's crosses, which involved E.angustifolium, fleischeri, and Dodonaei, 3 members of Chamaenerion, with eith E.hirsutum or E.montanum, were able to set seed. Such is exactly the case with the present crosses involving E.angustifolium and E.fleischeri, although in the crosses involving E.fleischeri a few good looking (but not viable) seeds were obtained. The irregularities that have been found in our crosses are abnormal division of the embryo leading to its irregular and undifferentiated shape, slow growth of the embryo, and disintegration of endosperm first into cellular structure and then its eventual dissolution. We did not examine microtome sections of the developing ovules and therefore it was not possible to see in details the abnormalities associated with the integuments and the nucellus. Nevertheless, lumps of cells of irregular shape and size were always found scattered under the coverslip after the squash and these were evidently hypertrophied nucellar cells.

Such abnormalities have not been observed in the seed development of the other crosses of Epilobium, except in the cross E.hirsutum x montanum in which there is irregular division of the embryo and in E.parviflorum x roseum in which the synergids occasionally divide and grow at the expense of the embryo. The usual feature associated with the seed failure in most of the crosses seems to be the slow growth of the embryo, most often accompanied with equally slow growing endosperm, and the inability of the embryo to get through one or the other critical stages of its differentiation.

Taking into account of the diploid constitution of the endosperm in Epilobium, in contrast to triploid or multiploid endosperm in other flowering plants, we looked into Brink and Cooper's (1947) explanation of seed failure in general and found, as has been pointed out while introducing the subject, that endosperm had no physiological advantage over either the embryo or the maternal tissue. There would be no worse interactions between the endosperm and the maternal tissue than those between the embryo and the maternal tissue. Consequently, somatoplastic sterility, expressed as histological abnormalities arising from greater genic disharmony within endosperm or between endosperm and maternal tissue, does not seem to be a satisfactory explanation of seed failure in Epilobium hybrids. Because the embryo and the endosperm are of the same genetic constitution, the interaction between them will be nil. The disintegration of each will, therefore, depend upon the degree of genic disharmony within each of the two, and not upon each other. Our results do show that both probably disintegrate independently and most often simultaneously.

Rietsema, Satina and Blakeslee (1953) explained seed failure in *Datura* crosses, in which almost all the peculiarities found in *Epilobium* have been observed, on the basis of physiological disturbances in the development of the hybrid seed. The disturbances are supposed to be due to lack of various enzymes in endosperm and embryo. As the seed failure usually occurs during various critical stages of growth and differentiation, we can safely presume that the genic disharmony is reflected in the physiological disturbances. As mentioned in the introduction, Michaelis (1954), on the basis of the differences observed between adult reciprocal hybrids, established that there was cytoplasmic as well as genic inheritance in *Epilobium*. As has already been noted, differences in seed development in reciprocal crosses are not very great, and it would appear that cytoplasmic genes have relatively little effect on seed incompatibility.

Hybrid Inviability and Weakness

Under this heading we include failure of the seed to germinate, early death of the hybrid seedlings and disturbed growth of the hybrid plants. Information about lethal and inhibited hybrids of Epilobium has already been given earlier. The barriers in this category work at various stages of the vegetative growth of the hybrid right from the germination of the seed onwards to the flowering stage.

As mentioned above, there is evidence, from the work of Michaelis, that cytoplasmic as well as genic factors are involved in both the viability and fertility of the hybrids. Differences in viability and vigour between reciprocal hybrids have been observed in our crosses a number of times, and these may be important in isolation.

On the whole, the hybrids between the cross-pollinated species are usually weak and inhibited, whereas the hybrids between the self-pollinated species (ecept those between E. Lamyi and E.obscurum, which die) are usually vigorous.

Hybrid Sterility

When the F1 hybrids are sterile, there will be no further intermixing between the species. In Epilobium hybrids, we have always found high sterility, except in hybrids between members of the section Synstigma. Many hybrids do not flower, e.g., E.fleischeri x angustifolium, E.hirsutum x montanum and E.lanceolatum x montanum. The weak constitution of many of the hybrids tells upon their flowering vigour and some of the hybrids bear only a few flowers. Most of the hybrids raised in the present study produce less than 50% good pollen (Table 26).

The seed set is still lower. Many of the hybrids are self-sterile and most of them can rarely set seed in nature. On backcrossing, these were able to set seed in good amount, but in nature this is unlikely to happen.

Sterility may be either genic or chromosomal. When sterility is chromosomal, there is distrubance in meiosis, but sometimes disturbance in meiosis can be caused by one or a few genes. In the few of the hybrids that have been meiotically examined, no irregularity in pairing was observed (Chapter V); similarly, Lewis and Moore (1962) and Raven and Moore (1964) have reported little meiotic irregularity in interspecific <u>Epilobium</u> hybrids.

The pollen is held together in tetrads in <u>Epilobium</u>. We attempted to count good and bad pollen per tetrad (as assessed on p. 106) in a few of the hybrids to find out if the pollen abortion was associated with genetic segregation at the time of meiotic division. [redacted] The hybrids with 25% of good pollen had the largest class of tetrads containing 3 bad and 1 good pollen; those producing 50% good pollen had the largest class of tetrads containing 2 bad and 2 good pollen; and those which were 75% pollen fertile had usually in their tetrads 1 bad and 3 good pollen. This gives a very cryptic suggestion that the pollen sterility might be due to some segregating factor.

Hybrid breakdown

As has been shown, some hybrids are sufficiently fertile to produce second generation offspring; but here too, recombination may produce plants which are inviable or of low fertility. The results of the experiments on the wild hybrid *E.montanum x obscurum* demonstrated this. Thus the possibilities for introgression are limited, though it would be interesting to look for it in the field. A further possibility is that selfing of a partially fertile F1 hybrid could produce F2, and by further selfing, other generations which, in time, would develop more or less pure lines which differed from the parents, and were isolated from them by a genetic barrier; these would be, potentially, new species. This kind of process has been demonstrated by Smith and Daly (1959) in *Nicotiana*; and it is at least conceivable that some of the *Epilobium* species which we have described arose in this way, rather than by divergent evolution in which interspecific hybridization was not involved. Time has not allowed us to test this hypothesis experimentally; but it should be pointed out that all the most viable and fertile offspring from the *montanum-obscurum* hybrid in fact resembled the *obscurum* parent and were not intermediates.

Summary

We may summarize this part of the discussion by saying that, in Epilobium, a wide variety of isolating factors, both external and internal, is involved; and the herbarium data indicate that, on the whole, isolation is effective and that hybrid swarms and introgressive hybridization are rare. It is interesting to observe that the internal isolating factors have an unusually wide range, including as they do, rate of pollen tube growth, seed-incompatibility, hybrid weakness, failure to flower and hybrid sterility at both first and second generations. In additon, both chromosomal and cytoplasmic genes are involved.

B. Bearing of the Results on Problems of Relationship

One of the main aims of this thesis was to elucidate relationships between the species of Epilobium included in our studies on the basis of the results of the interspecific crosses. The degree of intersterility or interfertility as demonstrated by the crosses indicates the degree of genetic relationship between the species concerned. However, taxonomic groupings are usually based on morphological resemblances rather than on genetic similarities. It is, therefore, interesting to see how far the two kinds of criteria indicating relationships can be correlated in Epilobium.

A conspectus of the important morphological characters of all the species investigated is given in Table 27. It will be noted from the Table that *E.angustifolium* and *E.fleischeri* have all characters in common and are separated from the rest of the species in having alternate leaves, short hypanthium, zygomorphic flowers, entire petals and pollen not held in tetrads. There is only one species, *E.nerterioides*, in the list which has entire petals but it differs widely from *E.angustifolium* and *E.fleischeri* in other characters. It is on this basis that the two species are placed in a separate group, Sect. *Chamaenerion* Tausch., by Haussknecht (1884). The fact that Sect. *Chamaenerion* is quite distinct has been recognized by all taxonomists and very often many of them have accorded it generic rank.

The rest of the species, placed in another group, Sect. *Lysimachion* Tausch., by Haussknecht, have been classified on the basis of a variety of characters, the main ones being (i) method of vegetative propagation, (ii) absence or presence of raised lines on stem, (iii) pubescence, (iv) entire or 4-lobed stigma, (v) papillose or glabrous seeds, and (vi) flower buds drooping or erect. Haussknecht's first step is to divide the Sect. *Lysimachion* into two, Division *Schizostigma* comprising species having 4-lobed stigmas, such as *E.hirsutum*, *E.parviflorum*, *E.luteum*, *E.montanum*, and *E.lanceolatum*, and Division *Synstigma* comprising all other species with entire stigmas, such as E.roseum, E.adnatum, E.Lamyi, E.adenocaulon, E.obscurum, E. palustre, E.alsinifolium, E.anagallidifolium, E.nerterioides and E.brevipes. The question can be asked, how far is this division of Sect. Lysimachion on the basis of only one pair of characters justified in expressing taxonomic relationship? Are Schizostigma and Synstigma really two distinct units of Sect. Lysimachion?

Looking at Table 28, which has been compiled from Table 27 by calculating the number of characters which pairs of species have in common, a number of interesting features regarding morphological relationship between species of Sect. Lysimachion are revealed.

(i) The two New Zealand species, E.nerterioides and E. brevipes, appear to be rather distantly placed from the rest of the species.

(ii) The alpine species E.alsinifolium and the marsh willow-herb, E.palustre, are also slightly removed from the other species. E.alsinifolium and E.palustre appear to be as distantly related between themselves as they are with others.

(iii) Each of the four species, mentioned above, is as closely related, on the whole, to Schizostigma as to the rest of Synstigma.

(iv) Within Synstigma, E.roseum, E.adnatum (including E.

tetragonum), E.Lamyi, E.obscurum and E.adenocaulon seem to be more closely related between themselves, but the degree of their relationship is not significantly higher than that between them and the members of Schizostigma.

Thus, on the whole, the division of Sect. Lysimachion into Schizostigma and Synstigma on the basis of a single character, viz., entire or 4-lobed stigmas is not supported if all the characters concerned are taken into consideration.

Relationship based on seed-incompatibility

The results from seed-incompatibility support the view that E.angustifolium and E.fleischeri are widely separated from the rest of the species of the genus. The crosses involving the two species failed to set seed. The causes of the seed failure have been discussed elsewhere and we have mentioned the irregularities associated with the seed collapse. In hybrids between more closely related forms, no abnormalities are evident in the earliest mitoses of the zygote and young embryo, but they occur at various later stages in development. In all the crosses involving these two species, the embryo showed numerous abnormalities and died much younger.

There was no seed set in the crosses involving E.nerterioides either. On this basis, this species should be regarded as a very distant relative of the European species of the genus. On the other hand, the moderately good seed set in the crosses involving E.brevipes goes against the morphological evidence.

Except in the crosses involving E.hirsutum, the seed set in all other crosses has been moderate to quite high. This should be regarded as evidence that all the species of Lysimachion, except E.hirsutum, are fairly close to each other. There has been a fair amount of seed set in the crosses involving a Schizostigma and a Synstigma species, very often rather higher than in the crosses between Schizostigma. This again does not support the distinction between Schizostigma and Synstigma.

Relationship based on the viability of the hybrids

In Epilobium, hybrid inviability and weakness are caused by both genetic and cytoplasmic factors (Michaelis, 1954), and great care should be exercised in assessing relationship between species only on the inviability of their hybrids, as a similar effect is produced in the interstrain crosses of the same species. Sometimes, one strain of a species may produce inviable or inhibited hybrids in a cross with another species, while another strain may produce perfectly viable hybrids with the same species.

The hybrids between E.hirsutum and E.montanum, between E. angustifolium and E.fleischeri, and between E.Lamyi and E.obscurum are weak and inviable. It has been a surprise to find hybrids between *E.Lamyi* and *E.obscurum* to be inviable, because usually our crosses between *Synstigma* species have produced vigorous hybrids. Also, we have seen that morphologically *E. Lamyi* and *E.obscurum* are very close to each other. As to *E. hirsutum*, several strains of this species are known (Michaelis, 1954) and these all give different results in different crosses.

However, if we look into the hybrids as a whole, we can make some assessment of relationship between the different species of Sect. *Lysimachion*. Out of 32 hybrids of normal vigour, 6 of them (which were very vigorous) were between *Synstigma* species, 10 of them between *Schizostigma*, and the rest were between *Schizostigma* and *Synstigma*. This indicates that, so far as hybrid viability is concerned, there is no significant difference between *Schizostigma* and *Synstigma*.

Relationship based on hybrid fertility

In general, the hybrids between *Synstigma* species are very fertile. It will be noted from the Table 26, which lists 37 hybrids according to their pollen fertility, that, out of 13 hybrids with pollen fertility above 75%, 7 are between *Synstigma*, only one between *Schizostigma* and the rest between *Schizostigma* and *Synstigma*.

We could sum up by saying that on the criteria of seed-incompatibility, hybrid vigour and hybrid fertility, the classification of Section *Lysimachion* into Divisions *Schizostigma* and *Synstigma* is not supported, and this conclusion is in agreement with the maphological evidence. No doubt a better classification of *Lysimachion* could be made, but this would need much further work in which all the species of the section were taken into account.

Relationship between Chamaenerion and Lysimachion:

We have already examined the relationships of *E.angustifolium* and *E.fleischeri* with other species of *Lysimachion* investigated in the present work on the basis of morphology and genetical data derived from their crosses. We would like now to examine in details how far *Chamaenerion* deserves treatment as a separate genus.

Raimann (1893) accepted *Chamaenerion* as a separate genus in Engler and Prantl, but gave no more reasons of its separation from *Epilobium* proper than what Haussknecht (1884) had given already for its sectional rank within the genus. Raven (1962a) has pointed out that none of the characters on which *Chamaenerion* is separated is peculiar to itself, although not all of them are combined in any other single member of *Lysimachion*. When all the members of *Chamaenerion* throughout the world are compared with the members of *Lysimachion* likewise taken from its entire range of the world distribution, they simply intergrade into each other. The species of Chamaenerion are distinct only when a few of them are present in a locality along with other distantly related members of Lysimachion. Raven (1962a) further states that this is the case in northern Europe where the members of Chamaenerion are sharply distinguished from those of Lysimachion.

On the basis of crossability, we have seen that Chamaenerion is well separated from Lysimachion because of the seed failure in their crosses. But it is worth considering that the 3 crosses involving E. fleischeri yielded some good looking seeds, and that 2 seeds of the cross E. fleischeri x hirsutum did germinate. What Raven says on morphological grounds can be true so far as the crossability and the production of successful hybrids are concerned. If the whole range of the members are brought into hybridization experiments, probably a few of them may hybridize. More experiments are required.

Considering the entire range of crossability within the genus, the species seem to intergrade from one extreme of zero crossability to the other where it is very high. If one regards crossability as a criterion for relationship between the species, it is a matter of personal judgement rather than anything else to draw a dividing line, just as it is in the case of morphological characters. As the fertility of hybrids between some of the species does not necessarily invalidate their specific recognition, there are no reasons why failure to cross should be considered as a criterion of generic relationship. For if so, how does one treat *E.nerterioides*, which failed to cross either with *E.montanum* or with *E.roseum*? Morphologically too, it is distantly related to the European species. In fact, there are many species of *Epilobium* in other parts of the world which are morphologically very distinct and still they are referred to as belonging to *Epilobium*, and Raven (1962b), considers *Chamaenerion* to be no more distinct than they are. In all probability, it appears that all had a common origin and they diverged from each other in morphological characters, to an extent depending upon their environment and breeding systems.

Relationship between the European and New Zealand species:

We have incorporated *E.nerterioides* and *E.brevipes* in our breeding experiments. Both species are native of New Zealand. The first species was introduced recently and is now naturalized in Great Britain and Ireland. The second species is cultivated in gardens and no information is available of its naturalization in any part of Europe. From the breeding point of view *E.nerterioides* has failed to cross with any of the European species, whereas *E.brevipes* has intercrossed and produced viable hybrids with *E.roseum* and *E.Lamyi*. Naturally, one is interested in the relationship between the European and the New Zealand species. It is possible that a few more, besides <u>E.brevipes</u>, of the New Zealand species may interbreed with the European ones; while others may not as is the case with <u>E.nerterioides</u>. It is interesting to know the extent of interbreeding within the New Zealand Epilobia. No published information is available, but Mr. Brockie (1963, personal communication) has been kind enough to inform me about the interspecific crosses he has made involving <u>E.nerterioides</u> as follows:

Description	No. of crosses made
Interfertile (i.e., producing F2)	16
F1 more or less weak (failing to flower)	5
Apparently good seed, failed to germinate	11
Failed to set seed	4
Total	36 crosses

This suggests that there is a wide range of crossability within the New Zealand species, just as we have found within the European ones and that they are certainly not one breeding group. Some of them have developed efficient internal isolating mechanisms.

On the basis of the fact that some species like <u>E.brevipes</u>

can inter-breed with the European species, an interesting probability arises that the two groups of species had a common centre of origin. They became geographically isolated without developing breeding barriers. The origin of isolating mechanisms, such as those evidenced in the crosses involving E.nerterioides, must have followed geographical isolation in due course of time. This supports Mayr's (1942) contention that geographic isolation must precede any other isolating barrier. This means too that the isolating mechanisms in the European species discussed earlier in this section, or the isolating mechanisms in the New Zealand species, or for that matter the isolating mechanisms in the species of Epilobium in any other parts of the world, have arisen independently and after the migration of the species from their centre of origin to the different parts of the world.

Another point worth mentioning is about the forms of species found in each of the two geographical regions. The New Zealand Epilobia are characterized by absence of species with 4-lobed stigmas. Some of the members with 4-lobed stigmas found in Europe, such as E.angustifolium, E.hirsutum and E.parviflorum, are widely spread and are considered advanced members of the genus (Raven, 1962a). Probably this indicates different rates of evolution in the two regions.

TABLE 27

A conspectus of the important morphological characters or the species of *Epilobium* investigated

	1 Stem erect	2 Plants usually 20 cm. or less	3 Leaves alternate	4 Leaves stalked	5 Leaves entire	6 Leaves orbicular to ovate	7 Stem villous or tomentose	8 Stem with glandular hairs	9 Stem with crisped hairs	10 Stem 2-4 raised lines	11 Hypanthium nil or small	12 Flowers zygomorphic	13 Petals entire not emarginate	14 Petal normally 79 mm.	15 Style deflexed	16 Stigma 4-lobed	17 Pollen Grain not in tetrads	18 Capsules glabrous	19 Seeds 1½ mm. or more	20 Stolon	21 Rosette	22 Root thick woody	23 Runner	24 Seeds papillose	25 Flower buds drooping
augustifolium	+	−	+	−	+	−	−	−	−	−	+	+	+	+	+	+	+	−	+	+	−	+	−	−	+
fleischeri	+	−	+	−	+	−	−	−	−	−	+	±	+	+	+	+	+	−	+	+	−	+	−	+	±
luteum	+	−	−	+	−	+	−	−	−	±	−	−	−	+	−	+	[illegible]	−	+	−	+	−	−	+	−
hirsutum	+	−	−	−	−	−	+	+	−	−	−	−	−	+	−	+	−	+	−	+	−	−	−	+	−
parviflorum	+	−	−	−	−	−	+	+	−	−	−	−	−	±	−	+	−	+	−	−	+	−	−	+	−
montanum	+	−	−	+	−	+	−	−	+	−	−	−	−	+	−	+	−	+	−	−	+	−	−	+	±
lanceolatum	+	−	−	+	−	−	−	−	+	+	−	−	−	±	−	+	−	+	−	−	+	−	−	+	+
roseum	+	−	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	−	+	−	−	+	+
adnatum tetragonum	+	=	=	=	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−
Lamyi	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−
obscurum	±	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−	−	+	−
adenocaulon	+	−	−	+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−
palustre	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	+	±
alsinifolium	+	+	−	+	−	+	−	−	−	±	−	−	−	+	−	−	−	±	−	+	−	−	−	−	+
nerterioides	−	+	−	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
brevipes	−	±	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−

TABLE 28.

Table showing number of characters similar between pairs of species, based on Table 27

		luteum	hirsutum	parviflorum	montanum	lanceolatum	roseum	adnatum (tetragonum)	Lamyi	obscurum	adenocaulon	palustre	alsinifolium	*nerterioides	*brevipes
SCHIZOSTIGMA	luteum	25													
	hirsutum	16	25												
	parviflorum	17	21	25											
	montanum	20	18	21	25										
	lanceolatum	21	19	19	22	25									
SYNSTIGMA	roseum	17	15	19	20	21	25								
	adnatum (tetragonum)	19	19	21	19	21	21	25							
	Lamyi	20	18	20	19	22	22	24	25						
	obscurum	17	20	19	18	19	19	23	22	25					
	adenocaulon	18	16	20	19	20	24	22	23	20	25				
	palustre	14	18	16	16	16	18	18	17	20	17	25			
	alsinifolium	17	17	13	19	19	17	17	18	19	16	16	25		
	*nerterioides	14	12	12	13	13	12	14	15	14	13	13	16	25	
	*brevipes	14	12	12	14	12	12	14	15	14	13	13	16	22	25

*New Zealand Species

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Production of artificial auto-tetraploids

(Colchicine treatments)

Seeds of *E. hirsutum*, *E. parviflorum* and *E. montanum* were germinated on moist filter paper in petri dishes. When the cotyledons emerged and diverged, the exuded drops of water were soaked off with filter paper and droplets of colchicine solution of the strengths 0.2%, 0.4% and 0.5% were applied to the apices in between the cotyledons with a fine brush. The application of colchicine was repeated after 24 hours. After the next 36 hours the treated seedlings were transferred to pots.

Results:

(i) *E. hirsutum*. 50 seedlings were treated with 0.2% colchicine; 35 seedlings with 0.4%; and 80 seedlings were treated with 0.5% soln. of colchicine. In all the cases, the first pairs of leaves were thick and the apices became swollen. All seedlings treated with 0.2% and 0.4% soln. gradually recovered and grew into normal plants, whereas the seedlings treated with 0.5% soln. showed many abnormalities. 50 seedlings died immediately after their transfer from the petri-dish to the pot; 12 appeared to have recovered; and 18 grew stunted, with thick leaves and never flowered.

(ii) *E. parviflorum*. 38 seedlings were treated with 0.2% soln. of colchicine; 68 seedlings were treated with 0.4% soln.;

and 61 were treated with 0.5% soln. The growth of the seedlings was very slow. All those which had been treated with 0.2% soln. recovered. Out of the seedlings treated with 0.5%, only 13 survived but they were stunted and had abortive flowers. Out of 61 seedlings treated with 0.4% soln., nearly 30 died and 3 showed some features attributed to polyploidy in the genus, such as larger flowers, thick leaves and 4-5 germpores in the pollen grains. The rest appeared to have recovered. The chromosome count was not made and hence the ploidy level could not be confirmed.

(iii) E. montanum. 62 seedlings were treated with 0.2% soln. of colchicine; 54 seedlings were treated with 0.4% soln.; and 32 were treated with 0.5% soln. The seedlings treated with 0.2% soln. recovered and grew into normal plants. 5 of the seedlings treated with 0.5% soln. survived and grew stunted with thick leaves, but 2 of these gave lateral branches which were normal and were diploid (as judged from their pollen grains with 3 germ-pores). Out of the seedlings treated with 0.4% soln., 26 died; 16 recovered; 4 were stunted in growth and did not flower; and 6 appeared to have become tetraploid. Their flowers were larger than those of the plants of the same stock; the leaves were thicker and darker with bigger stomata; and their pollen grains had 4-5 germ-pores. The chromosome count was also made.

Although no definite number of 2n = 72 could be clearly counted, it was certainly 64 ± 4.

Artificial polyploids have been produced in *Epilobium* and Michaelis (1954) gives a key to identification of ploidy level in the genus on the basis of the number of germ-pores. Haploid plants have pollen grains with 2 germ pores; diploid with 3 germ pores; and polyploids have pollen grains with more than 3 germ pores.

Douwes (1947) has made and described reciprocal hybrids between the diploid and tetraploid races of *E. hirsutum*, but no information about interspecific crosses on diploid-tetraploid level is available. In order to get a triploid interspecific hybrids between *E. angustifolium* (diploid) and *E. montanum* (tetraploid), reciprocal pollinations were made. Usually, the cross between *E. angustifolium* and *E. montanum* on the diploid level has failed; but it was expected that they might cross if either of the two was a tetraploid. This expectation was based on Valentine's success in crossing diploid *P. veris* with tetraploid *P. elatior*. However, the cross did not succeed in any direction and it appears that the isolating barriers between the two species are not removed by raising one of the parents to the polyploid level.

Douwes, H.	(1947)	Plasma, Polyploidie en Celgrootte. *Genetica*, *24*: 161.
Michaelis, P.	(1954)	Cytoplasmic inheritance in *Epilobium* and its theoretical significance. *Adv. Genet.*, *6*: 287.
Valentine, D. H. & Woodell, S. R. J.	(1963)	Studies in British Primulas X. *New Phytol*. *62*: 125.

Biosystematics of *Epilobium* by V. Thakur. Appendix 2.

Pollen-tube growth in interspecific crosses

Method: Pollinated styles with stigmas were fixed in 1:3 acetic alcohol at different periods after pollination. They were transferred into a tube containing distilled water and the tube was put into a water bath at 60°C. for about 5-6 minutes. Water was drained off the tube, the styles were split longitudinally into halves, and N HCl was poured in. The styles were left in N HCl at room temperature for 10 minutes and were then squashed in aceto-carmine. The pollen tubes, which were darkly stained in aceto-carmine, could then be traced down throughout the length of the style.

Crosses involving *E. nerterioides*: Styles of *E. mon~~at~~tanum* and *E. roseum*, pollinated with pollen from *E. nerterioides* have been examined 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours after pollination. The germination of pollen in both the cases appears to be somewhat slow; only a few pollen grains are seen to have germinated 4 hours after the pollination. In the styles fixed 24 hours and 48 hours after pollination, the pollen tubes could be traced down hardly more than half the length. Usually the styles of *E. montanum*, when selfed, show pollen tubes right down to the base within 4 hours after pollinati
Styles of *E. nerterioides* pollinated by *E. montanum* or *E. roseum*

have not been examined. They fall off quickly within 24 hours after pollination and also the capsules soon dry off. Obviously, the fertilization does not take place and this might be due to the inhibition of the pollen tube growth as in the reciprocal crosses.

Crosses involving E. angustifolium: Styles of both *E. angustifol* and *E. montanum*, when selfed, have shown pollen tubes right down to the base within 4 hours. When the species were crossed reciprocally, pollen tubes were traced down to only half the length of the style 5 hours after pollination in each. Styles fixed 12 hours after cross-pollination have shown pollen tubes throughout their length. It appears, therefore, that the rate of pollen tube growth within a foreign style in the cross between *E. montanum* and *E. angustifolium* is slowed down.

Biosystematics of Epilobium by V. Thakur. Appendix 3.

Grafting experiments

An attempt was made to graft *E. angustifolium* on *E. hirsutum*, and *E. hirsutum* on *E. angustifolium*. The attempt was made at two stages of growth. First, young plants of the two species were grown together in a pot and were grafted together. Second, the grafting was attempted between two new spring shoots of the old stocks. These shoots are fleshy and healthier and survive some injuries.

To graft the plants, the epidermis and cortex and a part of the vascular bundles from adjoining sides of the two plants were carefully removed with a sharp blade, and the two plants were laterally pressed so that their cut sides approximated together. They were bound together with a piece of cord at the cut portion. At regular intervals, the spot was moistened with water.

The grafting did not succeed in any case.