THE BREEDING RELATIONSHIPS OF SOME EUROPEAN EUPHRASIAE

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ABSTRACT

Previous chromosome counts on *Euphrasia* are summarized, and one new count is reported. Two chromosome numbers, $2n = 22$ and $2n = 44$ are known in the genus.

Euphrasia species are not apomictic but are fully self-fertile, producing viable seed. Crosses were carried out between different populations of the same species, and between different species, both alike and unlike in chromosome number. When chromosome numbers of the parents differed, partially developed seeds were produced, but no hybrids were obtained.

All other crosses gave a good seed set. However, when the parents were in different Subsections of the genus, seed development was variable. In most samples hybrid progeny were raised. Crosses within Subsections produced progeny ranging from almost normal in fertility (meiosis, pollen, seed and progeny) to somewhat sterile. Crosses between Subsections gave highly sterile progeny. Some crosses were carried through several generations; where fertility was initially reduced, it tended to rise in later generations.

The morphology of all the F_1 hybrids, and of later generations of three crosses, is partially described. The F_1 was usually intermediate in most but not all characters, while segregation in later generations suggested polygenic control of most characters. In one cross morphologically divergent lines were selected in later generations.

The flowers of *Euphrasia* are entomophilous but much selfing takes place, the amount probably depending on floral behaviour and flower size. Insect visits are rarely seen in nature, but can be effective in causing cross-pollination in cultivation. The occurrence of natural hybrids indicates the availability of cross-pollination to *Euphrasia* in Britain, but the frequency of hybrids is much affected by ecological conditions.

The ecological and distributional characters of the British species are summarized. Diploids are represented by fewer species and are less wide-ranging and less frequent in occurrence than tetraploids.

Sources of taxonomic difficulty in the genus, and the factors which may at present be maintaining the distinctness of the species are indicated. The appropriate taxonomic treatment of the *Euphrasiae* and their genecological status are briefly discussed.

CHROMOSOME NUMBER

The normal chromosome numbers of British *Euphrasiae* are $n = 11$ and $n = 22$. I have previously published a tabulation of the known chromosome numbers in the genus (Yeo 1954), and it is now possible to add to this list the count $n = 22$ for *E. salisburgensis* var. *hibernica* Pugsl., which I have obtained from metaphase II of meiosis. The count was made on a second-generation cultivated plant of a stock from near Lough Bunny, County Clare, v.c. H 9, Ireland (Tutin, 52129; my serial no. E230, specimen in **CGE**). Further counts are those of $n = 22$ for *E. stricta* Lehm. (Reese 1952), *E. brevipila*, *E. curta*, *E. rotundifolia* and *E. davidssonii* Pugsl. (Löve 1956). These authors state the origin of their material, though they do not say where voucher specimens are preserved. Instances of pairing failure at meiosis, leading to the occurrence of two univalents, have already been reported for a hybrid of *E. anglica* and for two of the three populations of *E. brevipila* investigated (Yeo 1954, p. 103; 1956, p. 263); the same irregularities have since been found in a sample of *E. brevipila* (from west of Bowes, N.W. York, v.c. 65, no. E247, specimens in **CGE** and Herb. Univ. Leicester) collected as *E. borealis*. One of the samples for which a count was given in my earlier paper (1954) was misidentified; it is no. E136, listed as *E. pseudokerneri*, and is in fact *E. confusa*.

The diploid species of *Euphrasia* belong to Subsection *Ciliatae*, Series *Hirtellae*, and are weakly differentiated morphologically from the tetraploids of the same Subsection, which are distributed in three other Series. The tetraploid number also occurs in Subsection

Angustifoliae, which is rather strongly separated morphologically from the other tetraploids. The only species counted in this Subsection is *E. salisburgensis*.

APOMIXIS

In order to find out whether apomixis could occur, I cut the anthers and stigmas out of some flowers just before they opened, using plants growing in a greenhouse (Yeo 1964). In a few cases, no observations could be made because of death or disease. In every other flower treated in this way, the capsule failed to develop normally or to dehisce; the capsules grew to only half to two-thirds the length of the calyx and did not swell. In nearly all cases, other capsules at the same node or a later one developed normally. This showed that enough time had been allowed for the unfertilized ovaries to develop before the observations ended. These unfertilized ovaries eventually died; one, 2 mm long, belonging to *E. anglica* (E76) was opened after death, 7½ weeks from the time of the excisions, and was found to contain eleven undeveloped ovules ·25 mm long or less. The number of flowers of each species for which this result was obtained is given in Table 1. Fertile capsules enlarge in about two weeks, becoming somewhat swollen and attaining approximately the length of the calyx; they dehisce after four to five weeks, and the seeds are usually about 1·5 to 2 mm long.

TABLE 1. *Euphrasia* species tested for apomixis.

	Number of capsules of treated flowers failing to develop
Tetraploid Species	
micrantha E112A	2
occidentalis E73	8
nemorosa E74	1
nemorosa E161	1
pseudokerneri E70	1
pseudokerneri E77	1
brevipila E81	3
Diploid Species	
anglica E71	12
anglica E76	4
rivularis E123	2
Total	35

I concluded from these results that apomixis does not play a significant part in the reproduction of *Euphrasia*. Later hybridizing work on these and allied species has not cast doubt on this conclusion.

SELF-FERTILITY

Self-fertility was tested by bagging the flowers of plants growing in the greenhouse, and in some instances assisting the transfer of pollen from anthers to stigma. In Table 2 the results are compared with those of open-pollination in the greenhouse. Extraction of seeds was facilitated by moistening the contents of the capsule first, wet seeds being much easier to handle than dry. Sometimes an unknown number of seeds appeared to have been lost from the capsules when they were first found to have dehisced. These capsules are not included in Table 2. The great majority of bad seeds produced by the selfed plants were very small empty ones, similar to those produced by the unpollinated capsule described earlier. Their occurrence is presumably due to failure of fertilization. As they are rather difficult to extract and detect I have put a dash in the table when I did not record any.

TABLE 2. Seed production with enforced self-pollination and with open-pollination, 1952–57.

	No. of capsules	*Total good seed*	*Total bad seed*	*Mean no. good seeds per capsule*
Self-pollinated				
micrantha E185A	3	28	8	9·3
occidentalis E192*	3	34	2	11·3
E561	10	151	6	15·1
nemorosa E608	10	104	4	10·4
E650*	7	94	13	13·4
confusa E601	4	26	—	6·5
pseudokerneri E42	2	24	—	12·0
E226*	10	99	6	9·9
E609*	6	50	4	8·3
salisburgensis E515 Bavaria	4	46	—	11·5
salisburgensis var. *hibernica* E230	2	13	3	6·5
anglica E180	8	82	3	10·3
E616	3	26	1	8·7
E663*	9	95	7	10·6
hirtella E559* Brittany	3	18	2	6·0
Open-pollinated				
pseudokerneri E42	38	274	—	7·2
anglica E71	28	265	—	9·5
E76	26	269	5	10·3
E663	4	24	20	6·0

*Pollination assisted in some or all of the flowers.

The remaining bad seeds from selfed capsules comprised a few that were undersized with much-reduced contents and two full-sized testas with no perceptible contents.

Full-sized but empty testas are the characteristic product of the pollination of diploid Euphrasias by tetraploids, and among the open-pollinated seeds recorded in Table 2 all the bad ones of E76, and 19 of those of E663, were of this type. Probably most of these seeds resulted from cross-pollination with tetraploids by insects. The production of good seed by open-pollinated E663 has thus been abnormally reduced. The table shows a high degree of self-fertility, approximately equal to fertility under open-pollination. The greatest number of good seeds in a single selfed capsule was 21 for *E. nemorosa*, 18 for *E. occidentalis*, 16 for *E. anglica* and 15 for *E. pseudokerneri*. Capsules with very few seeds are sometimes undersized, and their dehiscence is probably somewhat delayed.

Fifteen samples of seed resulting from self-pollination of eight species were later sown and germination took place in ten samples representing six species. Figures for some of these samples are given by Yeo (1961); germination ranged from one seed out of 31 to 24 out of 49. Poor germination or complete failure may not necessarily indicate infertility connected with selfing, as germination is often unexpectedly poor in *Euphrasia* (Yeo, l.c.).

CROSS-FERTILITY

Method

The anthers were removed from the flowers by cutting the filaments with a small pair of curved scissors. In large-flowered forms this was sometimes done as the flower was opening, but it is preferable to do it the day before the flower is due to open, and occasionally it was necessary to do it even earlier than this. Cuts in the corolla were usually unavoidable but they had no ill-effects. After extraction the anthers were examined and if they had begun to dehisce, or had been broken, the fact was noted, but the flower could still be used for cross-pollination provided there appeared to be little or no pollen on the stigma.

The resulting seeds were kept separate from those produced by flowers where there was not this slight possibility of selfing. The stigma was pollinated at the time of emasculation or up to three days after the flower had opened. Pollen was transferred on a mounted needle and a lens was used to see that the stigma was well pollinated. The flowers were protected by small paper bags, the lower ends of which were blocked by cotton wool.

Crosses were made between species alike and unlike in chromosome number and, among the latter, between species in the same Series and species in different Series. In addition, different populations of the same species were cross-pollinated. Of the species used, *E. pseudokerneri* (a British endemic) hybridises in nature with *E. nemorosa* and *E. occidentalis*, but shows a marked geographical and ecological separation from *E. brevipila*, *E. micrantha* and *E. scottica; E. anglica*, another British endemic, could not meet *E. hirtella*, which I do not believe occurs in Britain, but *E. confusa* and *E. micrantha*, which crossed spontaneously in cultivation, are known to cross in nature, as also are *E. brevipila* and *E. scottica; E. salisburgensis* var. *hibernica* appears to cross in nature with *E. nemorosa* and it could conceivably also come into contact with *E. occidentalis*.

Seed production and germination

The seed production from cross-pollinations between diploid and tetraploid species is given in Table 3; this includes figures for capsules where an unknown number of seeds was lost. However, there are few of these, because bad seeds, which predominated, do not fall out of the capsules as easily as good ones. Where there was definitely no selfing, good seed production was almost nil.

TABLE 3. Seed production from cross-pollination of species differing in chromosome number.

Euphrasias	*Selfing impossible* *No. of capsules*	*No. of seeds*		*Selfing possible* *No. of capsules*	*No. of seeds*	
		Bad	*Good*		*Bad*	*Good*
Diploid female × tetraploid male						
anglica × *brevipila* E76 × E81	1	13	0	3	15	6
anglica × *confusa* E76 × E179	1	13	0	2	22	2
E76 × E209	—	—	—	3	38	0
E135 × E179	—	—	—	5	41	2
anglica × *micrantha* E180 × E185A	7	79	0	3	38	0
anglica × *nemorosa* E76 × E154	1	16	0	1	14	0
E168 × E154	3	18	0	4	17	0
E135 × E196	—	—	—	2	10	10
E135 × E154	1	16	0	8	36	27
E180 × E196	12	151	1 or 2	2	32	0
anglica × *pseudokerneri* E76 × E77	2	25	0	1	13	0
E168 × E77	1	2	0	3	10	0
anglica × *scottica* E76 × E120	1	18	0	3	25	0
Totals	30	351	1 or 2	40	311	47
	(Mean per capsule = 11·7)			(Mean per capsule = 9)		
Tetraploid female × diploid male						
micrantha × *anglica* E185A × E493	2	16	0	—	—	—
nemorosa × *anglica* E129 × E76	—	—	—	5	0	0
E196 × E180	1	11	0	—	—	—
	2	?	0			
E474 × E493	10	14	0	5	10	0
nemorosa × *rostkoviana* E474 × E520	—	—	—	2	10	3
pseudokerneri × *anglica* E42 × E76	1	0	0	—	—	—
E77 × E76	—	—	—	2	0	0

The type of bad seed produced depended on whether the diploid or the tetraploid was the ovule parent. In the first case, the capsules developed normally and dehisced, though sometimes rather belatedly. As a rule they contained normal numbers of full-sized but empty, or nearly empty, testas. When the ovule parent was tetraploid, full-sized empty seeds were not produced. Many of the capsules were undeveloped, and doubtless contained minute empty ovules, which were seen in a number of cases though not usually counted. Occasionally, partially developed seeds were found; these had considerably undersized testas but some development of contents, though much less than in a normal seed, even when it nearly filled the testa. Usually several such seeds occurred in the same capsule which was itself partially developed (for example, the 24 bad seeds of *E. nemorosa* × *E. anglica*, E474 × E493, were produced by only three of the fifteen flowers pollinated). Seeds of this type were formed in all the crosses shown in the lower part of Table 3 except the two between *E. pseudokerneri* and *E. anglica* (they also appear in a number of later tables under the heading 'partly filled').

A possible interpretation of these results is that, when the diploid is female, fertilization takes place readily and seed-development begins; but although the testa reaches full size there is practically no development of the embryo and endosperm. The initiation of seed-development seems to stimulate the development of the capsule. When the tetraploid is the female, fertilization perhaps takes place more rarely, but when it does the development of seed-contents goes further than in the reverse cross. Perhaps, therefore, there is a better chance of getting a triploid from the cross with the tetraploid as female, although at first the reverse type of cross seemed more promising.

All the apparently good seeds from these pollinations were sown. Where selfing was thought to have been impossible one seed (of the cross *E. anglica* × *E. nemorosa*, E180 × E196) germinated but the seedling died at an early stage. A total of 58 seeds was sown where there was a possibility of selfing; of these 14 germinated and ten plants were raised, all of them like the seed-parents, and presumably resulting from accidental self-pollination. This failure to obtain hybrids between diploid and tetraploid species is not surprising in view of the fact that I have only once found a triploid in nature; this was a hybrid between *E. anglica* and *E. micrantha* (Yeo 1954, 1956).

The seed production in crosses between parents alike in chromosome number is given in Table 4. There are two types of cross here, namely those between two populations of the same species (*E. anglica*) and those between different species. The total seed production was moderate, and so also was the production of good seed, since only about one-ninth of all seeds were bad.

Most of the bad seeds that were produced were the minute empty ones described previously, but in the cross *E. occidentalis* × *E. salisburgensis* var. *hibernica*, E192 × E230, two of the bad seeds were half-filled and large; in the similar cross *E. nemorosa* × *E. salisburgensis*, E608 × E515, there was one large empty seed in a capsule in which the good seeds varied in size and shape; and in the reverse cross between these species, E515 × E608, one bad seed had contents but was only about half the normal thickness, while some of the seeds classed as good in the same capsule were also rather thin. In *E. anglica* × *E. anglica*, E663 × E649, four of the total of ten bad seeds were large empty ones. In the cross *E. pseudokerneri* × *E. micrantha*, E609 × E185A, most seeds appeared well-filled and looked normal, but it was noticed that they were distinctly smaller than artificially self-pollinated seeds of *E. pseudokerneri* and open-pollinated seeds of *E. micrantha;* they were, however, classed as good seeds.

Germination of these seeds is shown in Table 5. Although the crosses between Subsections (i.e. those involving *E. salisburgensis*) produced seeds of varying development, germination was quite good when compared with that of crosses within Subsection *Ciliatae.*

Meiosis in the hybrids

E. pseudokerneri × *E. nemorosa*, E42 × E154. Only one slide of pollen mother cell meiosis was made of this hybrid. Notes were made on 17 cells at various stages, but in none

TABLE 4. Seed production from cross-pollination between forms alike in chromosome number.

Parents *(female × male)*	*Selfing impossible* *No. of capsules*	*No. of seeds*		*Selfing possible* *No. of capsules*	*No. of seeds*	
		Bad	*Good*		*Bad*	*Good*
One species						
anglica × *anglica* E76 × E71	—	—	—	1	0	13
E76 × E168	2	0	38	1	0	16
E663 × E649	5	4	28*	3	6	36
Species in same Series						
anglica × *hirtella* E616 × E559	4	1	7*	4	0	15*
pseudokerneri × *nemorosa* E 42 × E154	2	0	13	2	0	15
E609 × E608	3	5	36	—	—	—
pseudokerneri × *occidentalis* E226 × E192	2	1	7*	4	0	15*
Species in different Series of same Subsection						
pseudokerneri × *brevipila* E70 × E81	—	—	—	1	0	5
E609 × E623†	1	0	10	—	—	—
brevipila × *scottica* E81 × E120	—	—	—	3	3	20
pseudokerneri × *micrantha* E609 × E185A	4	4	49	—	—	—
E699 × E185A	4	8	34*	1	6	8
pseudokerneri × *scottica* E42 × E120	1	0	8	1	0	11
Species in different Subsections						
salisburgensis var. *hibernica* × *occidentalis* E230 × E192	7	7	28*	3	4	19
occidentalis × *salisburgensis* var. *hibernica* E192 × E230	6	4	42*	4	2	24
salisburgensis × *nemorosa* E515‡ × E608	—	—	—	2	4	22
nemorosa × *salisburgensis* E608 × E515‡	1	1	11	2	1	20
Totals	42	35	311	32	26	239
	(Mean per capsule = 8·2)			(Mean per capsule = 8·3)		

*Some other seeds lost, or probably lost. †From Germany. ‡From France.

TABLE 5. Germination of hybrid seed, and plants raised from it.
(Parents alike in chromosome number)

	No. of seeds sown	*No. of seedlings*	*Plants raised* *hybrid*	*non-hybrid*
Inter-population crosses of *E. anglica*	139	30	25	0
Interspecific crosses within Subsection Ciliatae	203	45	28	0
Interspecific crosses between Subsections Ciliatae and Angustifoliae	159	30	12	1

was the interpretation quite clear. No definite irregularities were seen, and it is possible that meiosis was normal in this hybrid. It seems clear that, if there were any irregularities, they could not have involved more than one bivalent.

E. pseudokerneri × *E. scottica*, E42 × E120. About 65 cells derived from three plants were examined. The preparations were slightly better than those of the preceding hybrid. It was rarely possible to get a complete interpretation of a cell, but it seems that meiosis may occasionally have been normal, as it was not always possible to detect irregularities. Usually, however, univalents were visible at Metaphase I (Fig. 1), most frequently two in number, and six at the most. Very little was seen of other stages of meiosis, but in one Anaphase II a group clearly consisting of 22 chromosomes was counted, and at Telophase

II it was noted in one cell that two chromosomes had not quite joined their nearest reforming nuclei, while in another more advanced cell there were no stray chromosomes.

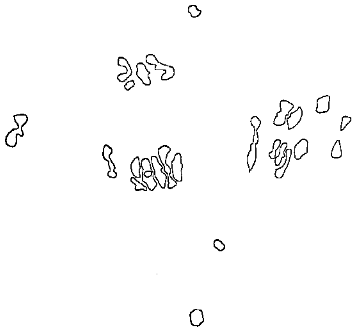

Fig. 1. Metaphase I of meiosis in the F_1 hybrid *Euphrasia pseudokerneri* × *E. scottica*, E42 × E120, showing 20 bivalents and 4 univalents. × 1100.

E. salisburgensis var. *hibernica* × *E. occidentalis*, E230 × E192. Notes were made on about 110 cells derived from two plants. Most of the preparations were good, and definite interpretations of some cells were obtained. All stages of meiosis were seen. There were always many univalents present at Metaphase I (Fig. 2); these were counted as accurately as possible, and the results for 40 cells were as follows:

1 cell had 12 univalents
4 cells had 16 ,,
6 ,, ,, 18 ,,
10 ,, ,, 20 ,,
11 ,, ,, 22 ,,
7 ,, ,, 24 ,,
1 cell ,, 28 ,,

Where an odd number of univalents was visible, one was added to the count, on the assumption that they always occur in pairs. When the chromosomes resulting from the disjunction of bivalents were congregating at the poles at late Anaphase I, some of the univalents came into the equatorial region and divided (Fig. 3), while others were to be seen in the peripheral region and probably did not divide. Finally, all chromosomes joined one or other of the Telophase nuclei.

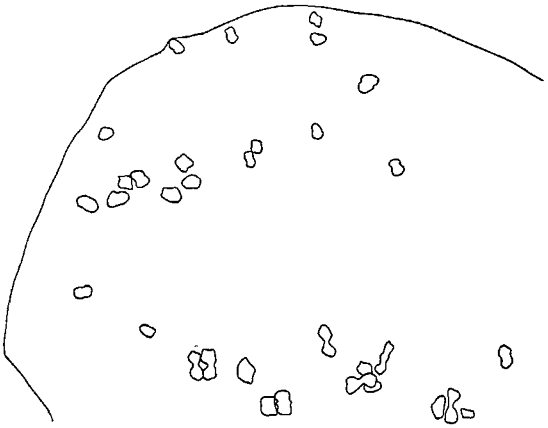

Fig. 2. Metaphase I of meiosis in the F_1 hybrid *Euphrasia salisburgensis* var. *hibernica* × *E. occidentalis*, E230 × E192, showing 12 bivalents and 20 univalents (mostly carried away from the equatorial region in squashing the cell). × 1100.

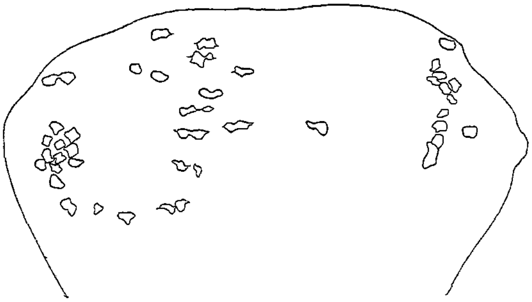

Fig. 3. Anaphase I of meiosis in E230 × E192. Univalents dividing in the equatorial region; the rather indefinite outlines which the univalents showed are not indicated by this method of drawing. × 1100.

At Metaphase II the chromosomes that divided at the first division of meiosis did not line up on the equator but remained scattered and appeared as laggards at Anaphase II (Fig. 4). However, nearly all the laggards eventually joined a nucleus (Fig. 5). Such an irregular meiosis can rarely lead to the production of a pollen grain with a normal set of chromosomes, and any grains which contain 22 chromosomes may well lack some members of the normal set and possess others in duplicate. Some counts were made at Anaphase II and Telophase II which confirmed the inconstancy of the final chromosome numbers. In one cell there were probably 24, 21, 22, and 20 chromosomes in the four nuclei, plus one stray chromosome; other chromosome counts for individual nuclei were 16 or 17, 18, 22 or 23, 24, and for cells at Anaphase II, in which the nuclei could only be counted in pairs, 41 + 47, 39 + 49, 36 + 52, 40 + 48, 42 + 46.

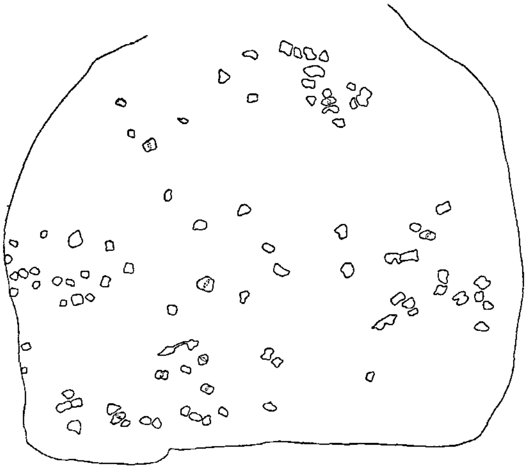

Fig. 4. Anaphase II of meiosis in E230 × E192. (As the figure shows 89 bodies, it may be that some other matter had been mistaken for a chromosome.) × 1100.

Production of normal pollen in the hybrids

Pollen counts of hybrids and their parents were obtained by breaking up the anthers in cotton-blue in lactophenol. The numbers of good and bad grains were counted, the good ones being rounded and darkly stained, and the bad ranging from minute to fairly large without contents, or having darkly stained contents but being markedly undersized. These counts were made during July and August 1954; on 6 October 1954, it was discovered

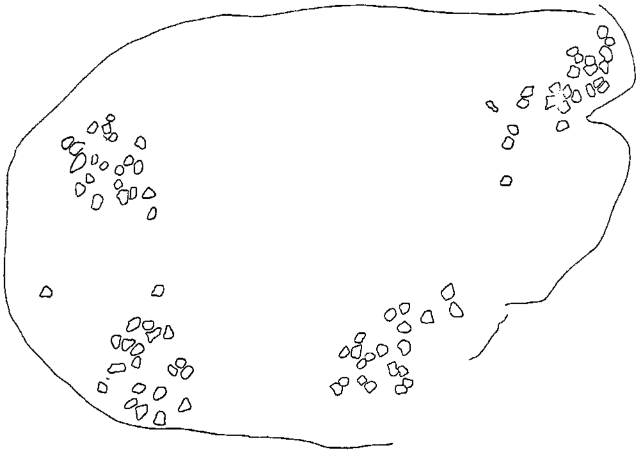

Fig. 5. Late Anaphase II of meiosis in E230 × E192. Nearly all the chromosomes have joined one of the re-forming nuclei. × 1100.

TABLE 6. Production of normal pollen in species and hybrids of *Euphrasia*.

	Production of normal pollen (approx.)
	per cent.
Parents	
pseudokerneri E42 × E70	100
E226	95
occidentalis E192	? 100
salisburgensis var. *hibernica* E230	? 100
Cross within one species	
anglica × *anglica* E76 × E168	100
Crosses within one Series	
pseudokerneri × *nemorosa* E42 × E154	? 100
pseudokerneri × *occidentalis* E226 × E192	? 90–95
Crosses between Series	
pseudokerneri × *brevipila* E70 × E81	85
pseudokerneri × *scottica* E42 × E120	*c.* 70
brevipila × *scottica* E81 × E120	85
micrantha × *confusa* E185A × E183C	85
Cross between Subsections	
salisburgensis var. *hibernica* × *occidentalis* E230 × E192	17–24

that all the counts had been lost and all that remained was a list of pollen samples taken. However, I could remember most of the counts approximately and at once wrote them down. They are given in Table 6.

In the hybrid *E. pseudokerneri* × *E. scottica* pollen counts were first made from three plants; two of them produced about 70 per cent of normal pollen, and the third produced considerably more. Additional pollen counts were made on anthers collected about two weeks later from two of these plants in order to see whether the discrepancy would be repeated. Both plants gave very much lower proportions of normal pollen than before.

It seemed, therefore, that the production of normal pollen was lower at the later date. If this was so, pollen fertility must fluctuate and one must not expect to assign fixed values to the production of normal pollen in hybrids. Probably, counts showing little or no bad pollen in plants that are not hybrids are more reliable. Table 6 shows a fairly good correspondence between pollen fertility and the affinity of the hybrid's parents although, in view of what has been said, such a conclusion must be regarded as tentative.

Finally, it should be pointed out that these counts are of visually normal pollen, and that the correlation between this and physiologically good pollen is not known.

TABLE 7. Seed production and germination of seed of F_1 hybrids when selfed, open-pollinated or back-crossed, 1954–1958.

Parentage of hybrid	*Polli-nation*	*No. of plants of the hybrid*	*No. of capsules*	*Seeds produced*			*No. of good seeds per capsule*	*Germi-nation*
				Good	*Small empty*	*Large empty, or partly filled*		
Species in same Series								
pseudokerneri × *nemorosa*								
E609 × E608	selfed	1	2	15	5	0	7·5	+
E609 × E608	× E609♀	1	4	24	3	1	6	+
pseudokerneri × *occidentalis*								
E226 × E192	selfed	1	6	66	1	3	11	+
anglica × *hirtella*								
E616 × E559	selfed	1	23	57	70	7	2	+
E616 × E559	× E616♂	1	1	5	0	5	5	+
Species in different Series, same Subsection								
micrantha × *confusa*								
E185 × E183	selfed	1	28	227	5	7	8	—
pseudokerneri × *brevipila*								
E70 × E81	selfed	1	8	85	2	3	10·5	—
E609 × E623	selfed	2	17	174	19	5	10	+
E609 × E623	× E699♀ (*pseudokerneri*)	1	4	45	2	0	11·3	+
pseudokerneri × *scottica*								
E42 × E120	selfed	3	13	100	34	18	7·5	—
pseudokerneri × *micrantha*								
E609 × E185	selfed	1	17	100	99	4	6	+
E609 × E185	× E185♂	1	4	23	24	1	6	+
E609 × E185	× E609♀	1	4	18	26	0	4·5	+
E609 × E185	× E699♀	1	1	9	2	0	9	—
Species in different Subsections								
salisburgensis var. *hibernica* × *occidentalis* E230 × E192	selfed	2	13	8	4	2	<1	—
E230 × E192	open-poll.	1	13	18	14	3	1·5	+
salisburgensis × *nemorosa*								
E515 × E608	selfed	3	29	4	many	2	<1	—
E515 × E608	open-poll.	4	17	14	c. 100	19	<1	+
E515 × E608	× E701♀ (*nemorosa*)	2	3	1	7	3	<1	+*
nemorosa × *salisburgensis*								
E608 × E515	× E608♀	1	4	0	10	9	0	+*
E608 × E515	open-poll.	1	21	7	many	2	<1	+*

*Including seed from additional capsules where accidental selfing was possible.

Seed production and germination in hybrids

Seed production and germination is the conclusive test of fertility in hybrids, and this is shown in Table 7; pollination was artificially assisted in nearly all flowers covered by the table. This shows that normally developed seed was obtained from the F_1 of all crosses. The number of good seeds per capsule was quite high in all but one of the hybrids whose parents belonged to the same Subsection. The exception was *E. anglica* × *E. hirtella* and it is difficult to account for its infertility. The seeds produced by five open-pollinated capsules of this cross were also counted: these capsules were similarly infertile and contained only one to six seeds each. However, most of the estimates of F_1 fertility in this work are based on single individuals and could therefore easily be biased. The somewhat low rates of seed production in *E. pseudokerneri* × *E. scottica* and *E. pseudokerneri* × *E. micrantha* and the low rate in *E. anglica* × *E. hirtella*, are associated with a large proportion of small empty seeds similar to those found in unpollinated capsules; their occurrence here could be caused by inviable embryo sacs, which would be equivalent to inviable pollen grains. The production of large empty or partly filled seeds by hybrids (which must be due to inviability after fertilization) is probably also a reflection of sterility in the F_1, as it is particularly noticeable in *E. pseudokerneri* × *E. scottica*, which would be expected to show some effects of its rather unbalanced meiosis. *E. micrantha* is closely related to *E. scottica* and produced a slightly more sterile hybrid with *E. pseudokerneri* than *E. scottica* did. However, *E. pseudokerneri* (E609) itself had a rather low fertility (Table 10).

The crosses between species of different Subsections were very much more sterile than the others. The plants produced so few seeds that it was necessary to collect seeds resulting from open-pollination, as it was feared there might otherwise be no progeny at all. In E230 × E192 many of the capsules covered by Table 7 were quite undeveloped and failed to dehisce; those with good seeds, large empty or partly filled ones were more or less well-developed, while those which contained only small empty seeds were more or less undeveloped at first, but later in the season even this class of capsule developed quite well. Capsule development in E515 × E608 was very much the same, but the sterility was even greater. Both crosses gave more seed under open-pollination than when selfed.

It can be seen from Table 7 that several large samples of seed failed to germinate; since F_2 seed of comparable hybrids germinated in other years, these failures are most

TABLE 8. Seed production and germination of seed of generations later than F_1, when selfed (cont. in Table 9).

Hybrid generation and year grown	*No. of plants*	*No. of capsules*	*Seeds produced*			*No. of good seeds per capsule*	*Germination*
			Good	*Small empty*	*Large empty, or partly filled*		
pseudokerneri × *nemorosa* E609 × E608							
F_2 (1958)	1	4	19	23	1	5	+
back-cross to E609 (1958)	1	9	58	43	0	6·5	+
anglica × *hirtella* E616 × E559 F_2 (1958)	4	11	51	11	1	5	—
pseudokerneri × *brevipila* E609 × E623							
F_2 (1958)	1	12	113	36	4	9	+
F_3 (1959)	1	12	109	25	5	9	+
pseudokerneri × *micrantha* E609 × E185							
F_2 (1959)	2	19	101	82	1	5	+
back cross to E185 (1959)	1	7	46	15	7	7	+
back-cross to E609 (1959)	1	6	49	25	0	8	+
F_3 (1960)	1	3	24	13	5	8	+
	1	9	48	42	3	5	+
back-cross to E185 selfed (1960)	1	11	128	32	3	12	+

probably due to unfavourable conditions of seed storage. Three of the F_2 seedlings of *E. pseudokerneri* × *E. occidentalis* were very pale yellow and died about a week after they had germinated; this is the only occasion on which I have seen albino Euphrasias, either among hybrids or among species.

Seed production and germination in generations later than the F_1 is shown in Tables 8 and 9. Production in *E. pseudokerneri* × *E. nemorosa* was lower than in *E. pseudokerneri* × *E. brevipila*, as it was in the F_1; it was also still rather low in *E. anglica* × *E. hirtella*. In the *E. pseudokerneri* × *E. micrantha* goup, fertility tended to rise after the F_2, but one of the F_3 plants gave a low value and consistently had few (4–8) and large seeds in its capsules. The back-cross to *E. micrantha* selfed produced small and numerous seeds ((6–) 10–16 per capsule). In this cross, one plant of the F_2 in 1959 was male-sterile, and a plant of the F_3 in 1960 at first produced little pollen and later none. The cross *E. salisburgensis* × *E. nemorosa* is covered by Table 9. The fact that some generations were grown from open-pollinated seed means that further hybridization could have taken place, and evidence that it did so is given later. The most likely sources of pollen, judging by the characters of the progeny, were *E. pseudokerneri* and its hybrids. These belong to Subsection *Ciliatae* and are genomically similar to *E. nemorosa*. The accidental cross-pollination of the F_1 *E. salisburgensis* × *E. nemorosa*, and of the reciprocal hybrid, would thus be similar in effect to a back-cross. The figures for all the generations in Tables 8 and 9 show a gradual increase in fertility.

TABLE 9. Seed production and germination of seed of generations later than F_1 (cont. from Table 8).

Generation	*Polli-nation*	*No. of capsules*	*Seeds produced*			*Good seeds per capsule*	*Seeds sown*	*No. germi-nating*
			Good	*Small empty*	*Large empty, or partly filled*			
salisburgensis × *nemorosa*								
E515 × E608 'F_2', from F_1 open-poll. One plant	open-poll. 1958	31	1	*c.* 25	4	<1	1	0
E515 × E608 'F_2', from F_1 open-poll. Two plants	× E608♀ 1958	6	4	17	0	<1	4	2
E608 × 'F_2' plant 6	selfed 1959	13	47	>83	5	3·5	47	29
E608 × 'F_2' plant 6	open-poll. 1959	24	48	>146	17	2	48	23
E608 × 'F_2' plant 7	open-poll. 1959	25	21	>97	5	<1	22	12
E608 × 'F_2' plant 4	open-poll. 1959				even less fertile			
Selfed progeny of plant 6 above. One plant	selfed 1960	14	131	74	3	9·5	128	48
nemorosa × *salisburgensis*								
E608 × E515 'F_2', from F_1 open-poll. One plant	selfed 1959	13	15	>67	3	1	14	11
nemorosa × *salisburgensis*								
E608 × E515 'F_2', from F_1 open-poll. One plant	open-poll. 1959	8	11	46	4	1·5	11	7

Occasionally the highly sterile hybrids involving *E. salisburgensis* produced some seeds which, though rather thin, and thus approaching the 'partly-filled' category, were thought to be worth sowing. The thinness of these seeds was presumably due to some genetical defect; other seeds, apparently without any such defects, were exceptionally large, doubtless because they received extra nourishment through being so very few in each capsule. Eleven of the seeds produced by E515 × E608, open-pollinated in 1957, were sown; of these, six were thin and the rest large. They produced four particularly large seedlings and one particularly small one; it seems likely that the small seedling arose from one of the thin seeds and that such seeds are occasionally viable.

During the period when these hybrids were growing, some of the parent populations were maintained by enforced self-pollination, and detailed records were kept of the bad seeds produced. The results are shown in Table 10. It will be seen that *E. pseudokerneri* (E609) was consistently rather infertile, as mentioned earlier. Production of large empty seeds is appreciable though quite low, while partly filled seeds are very rare; this applied also in interspecific pollinations and in the resulting hybrid generations.

TABLE 10. Seed fertility in the parents of *Euphrasia* hybrids

Species		*No. of capsules*	*Seeds produced*				*No. of good seeds per capsule*
			Good	*Small empty*	*Large empty*	*Partly filled or under-sized*	
pseudokerneri E609,	1957, 2 plants	5	27	12	1	0	5
	1958, 2 plants	20	143	80	6	1	7
	1959, 1 plant	7	59	26	1	0	8
pseudokerneri E699,	1957, 1 plant	9	82	9	2	0	9
	1958, 1 plant	5	57	3	1	0	11
nemorosa E701,	1957, 2 plants	16	213	3	0	1	13
nemorosa E608,	1958, 2 plants	14	180	11	5	1	13
anglica E616,	1958, 1 plant	11	140	3	3	0	13

THE MORPHOLOGY OF THE ARTIFICIAL HYBRIDS

Herbarium specimens have been preserved of all the hybrids raised, and in nearly every case specimens of the parents, grown under similar conditions, have also been kept. In addition, flowers and leaves of most plants have been mounted on cellulose tape and photographed by contact printing. A disadvantage of the herbarium specimens is that all plants used as seed parents were pressed much later than their sister plants and later than the best time for showing their characters. Their mounted flowers and leaves are, on the other hand, quite comparable with those of the other plants. This material shows that F_1 hybrids were usually intermediate between the parents, or exhibited combinations of characters of both parents, but that they were occasionally outside the range of both parents in certain characters.

Figs. 8 and 9 and Tables 11–15 show intermediacy of hybrids in number and direction of branches, length of internodes, size and shape of corolla, and node and date of flowering. When plants with glandular hairs on the leaves were crossed with eglandular (or almost eglandular) plants, some progeny were eglandular, some were intermediate in frequency of glands, and some had about as many glands as the glandular parent; this applied in full

TABLE 11. *E. anglica* × *E. hirtella*, comparison of flowers.

	Angle a (degrees)	*Angle b (degrees)*
E. hirtella, E559, 4 plants	13–18	60–80
F_1 hybrid, 2 plants	22–29	65–95
E. anglica, E616, 4 plants	35–45	85–100

TABLE 12. *E. pseudokerneri* × *E. nemorosa*.

	No. of plants	*Node of last branch*	*Node of first flower*
E. nemorosa, E608, 1958–9	7	8–12	12–16
F_2 hybrid, 1958–9	3	17	18–24
Back-cross to E609, 1958	3	13–16	18–19
E. pseudokerneri, E609, 1958–9	10	11–25	20–26

TABLE 13. Node of first flower (F) and number of primary branching nodes (B) of *Euphrasia* hybrids and parents.

	pseudokerneri E42	*pseudokerneri* × *brevipila* E42 × E81	*pseudokerneri* × *scottica* E42 × E120	*brevipila* × *scottica* E81 × E120
No. of plants	5	3	10	3
Range of F	13–16	7–9	7–9	6–7
Range of B	3–10	3–5	2–4	0–1

TABLE 14. *E. pseudokerneri* × *E. brevipila*, E609 × E623.

	No. of plants	*Start of flowering*	*Node of first flower*	*No. of primary branching nodes*
E623, 1956	7	Late July–early Aug.	10–12	0–4
E609, 1956	8	Mid-Aug.	16–21	6–14
E609, 1957	6	Late Aug.–early Sept.	15–23	(3—) 9–12
E609, 1960	4	—	17–19	3–7
F_1 hybrid, 1957	3	Late July–early Aug.	13–15	4–7
F_2 hybrid, 1958	7	—	10–14	1–6
F_4 hybrid, 1960	6	—	11–12	1–6
Back-cross: E699* × (E609 × E623), 1958	4	—	14–17	4–7
E699*, 1957–8	3	—	17–22	7–11

**E. pseudokerneri* of different origin from E609.

TABLE 15. Length of lower corolla lip, including tube, of hybrids and parents (in arbitrary units).

		pseudokerneri E609	*Back-cross to* E609	F_2 *hybrid*	*Back-cross to* E185	*micrantha* E185
1956–8	number of plants	18				12
	length	33–50				21–27
1959	number of plants	15	5	15	5	2
	length	39–50	33–45	29–38	27–35	24–26

to the cross *E. pseudokerneri* × *E. occidentalis* (E226 × E192), where *E. occidentalis* is the glandular parent; it applied in part to *E. pseudokerneri* × *E. brevipila* (E70 × E81), where eglandular and sparsely glandular hybrids were produced, and also to *E. brevipila* × *E. scottica* (E81 × E120), where freely glandular and sparsely glandular plants were produced (*E. brevipila* being the glandular parent). An example of parental and hybrid leaf differences is seen in Fig. 11, and flower shape differences are seen in Figs. 6, 7 and 10. Leaf shape is fairly characteristic for species, though naturally each species shows some variation. Flower size is also characteristic within limits but the details of flower shape characterize populations rather than species; flower shapes are, therefore, useful as a form of easily presented evidence that hybrids have been obtained when the parents represent closely similar species, or populations within a species. Although marked differences from year to year in the flower shapes of wild plants growing at particular localities were observed by Wilkins (1963), this character has always appeared to me to be a reliable one in cultivated plants. I think that shape variations associated with size variations, such as described by Wilkins, have occurred in my cultures, but they did not seem to be great enough to affect the usefulness of details of corolla shape for purposes of identification.

Some details of the hybrid morphology will now be given, as evidence that hybrids were in fact raised, and in some cases to show the changes over more than one generation of hybrids.

E. anglica, inter-population hybrids (E76 × E168) and (E76 × E71): no plant of the female parent was raised under the same conditions as the F_1, but both hybrids differed from the selfed progeny of the pollen parents in habit, leaf shape and flower shape (Fig. 6).

E. anglica × *E. hirtella* (E616 × E559): in *E. hirtella* the plants matured earlier and the branches developed later than in *E. anglica;* the hybrids were intermediate in these characters. The difference in flower shape was rather marked and the intermediate nature of the progeny is very clear from Fig. 7 and from Table 11, in which measurements are given for the angles which are explained in Fig. 7.

E. pseudokerneri × *E. nemorosa:* the plants of this hybrid raised in 1954 (E42 × E154) were more robust and larger leaved than the parental population of *E. nemorosa* (thus approaching *E. pseudokerneri*, as it grew in my cultures) and had more erect branches than *E. pseudokerneri* (being about the same as *E. nemorosa* in this respect). The flowers were

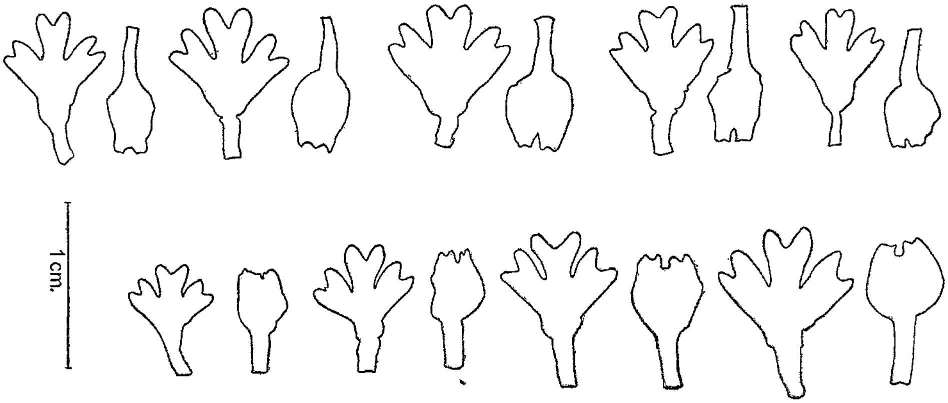

Fig. 6. Flowers of *Euphrasia*. The upper lip and tube, though often distorted in preparation, show overall length of flower and serve as a guide to the relative size of the lower lip, the shape of which is not distorted. Above, flowers of three populations of *E. anglica* and hybrids between them. Left to right, E71 (v.c. 11); E76 × E71; E76 (v.c. 17); E76 × E168; E168 (v.c. 55). Below, left to right, *E. nemorosa*, E608 (1956); *E. pseudokerneri* × *E. nemorosa*, E609 × E608, F_1 (1957); F_1 back-crossed to E609 (1958); *E. pseudokerneri*, E609 (1957). × 2·2.

intermediate in shape between those of the parents but about the same size as those of *E. nemorosa*, which has smaller flowers than *E. pseudokerneri*. When this cross was repeated in 1956 (E609 × E608) the parents and hybrids showed much the same characters as before, although the features of the hybrid are known chiefly from the F_2 because only one very weak plant was raised of the F_1 generation. Some numerical data on this cross are given in Table 12, where the F_2 and the back-cross to *E. pseudokerneri* are alike in the characters considered; in other characters, seen in the dried plants, the back-cross approaches very closely *E. pseudokerneri*, and its flowers are closely similar to those of this species (Fig. 6). The F_2 and the back-cross were each selfed and the progeny differed in much the same way as their parents.

E. pseudokerneri × *E. occidentalis* (E226 × E192): some of the hybrids raised are shown in Fig. 8. Four F_1 and seven F_2 plants were raised; the F_2 plants shown in the figure represent the extremes of the variation.

E. pseudokerneri × *E. brevipila: E. brevipila* differs little from *E. pseudokerneri* in its flowers and upper leaves, but it has fewer branches and begins to flower earlier and at a lower node. This cross was carried out twice, and the behaviour of the plants in the characters just mentioned can be seen from Tables 13 and 14. No specimens of *E. brevipila* (E81), grown under comparable conditions to the other plants of the earlier cross (E42 × E81), were available. The *E. brevipila* for the second cross (E609 × E623) came from north-east Germany; its branches were particularly late in development, but their growth had started sufficiently for them to be counted in the dried specimens. The plants of E609 grown in 1960 were rather weak and branched less freely than those grown in other years and the F_3 hybrid generation is omitted from Table 14 as it consisted of only one plant.

E. brevipila × *E. scottica* (E81 × E120): no suitable specimens of either of the parents of this hybrid are available for comparison, but comparisons with other hybrids involving the same parents are given in Table 13. In shape, and in their small size, the flowers of the hybrids showed very strongly the influence of *E. scottica*.

E. pseudokerneri × *E. scottica* (E42 × E120): specimens involved in this cross between two widely different species are shown in Fig. 9 in which E120 is represented by a later gathering of wild-collected specimens from its original locality. In addition to the differences that can be seen in the figure, the hybrids differed from *E. pseudokerneri* in their cuneate-based floral leaves (not truncate) with fewer teeth, and in their small flowers; these differences were in the direction of *E. scottica* (see also Table 13).

E. micrantha × *E. confusa* (E185 × E183): a seedling grown in 1954, resulting from open-pollination of *E. micrantha* in 1953, proved to be this hybrid. It was particularly

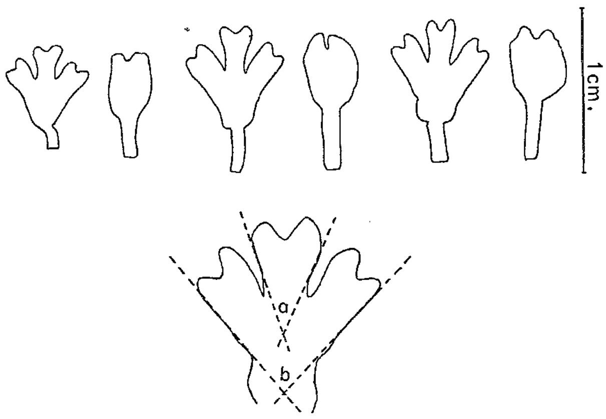

Fig. 7. Flowers of *Euphrasia*. Above, left, *E. anglica*, E616; right, *E. hirtella*, E559; centre, E616 × E559, F_1; all 1954. × 2·2. Below, diagram to explain angles *a* and *b*, used in Table 11.

Fig. 8. Pressed specimens of *Euphrasia*. Right to left. *E. pseudokerneri*, E226 (1955); *E. occidentalis*, E192 (1953); E226 × E192, F_1 (1954); F_2 (1955), two plants. × $\frac{1}{2}$.

Fig. 9. Pressed specimens of *Euphrasia*. Left, *E. pseudokerneri*, E42 (1954); right, *E. scottica*, E659, two wild plants from locality of E120 (1956); centre, E42 × E120, F_1 (1954). × $\frac{1}{2}$.

interesting in that the flowers were yellow—a character which appeared to be dominant in inheritance, and which it must have received from the pollen parent, the rare yellow-flowered *E. confusa* forma *confusa* which I had grown in 1953.

E. pseudokerneri × *E. micrantha* (E609 × E185): the F_1 generation in this cross consisted of a single plant raised in 1958; this was selfed and back-crossed to both parents, and subsequently fresh generations were raised each year until 1961. Three successive generations of the back-cross to *E. micrantha* were grown; these remained fairly uniform and were morphologically close to *E. micrantha*, but they tended to be larger and bushier and had slightly larger flowers (Fig. 10; the leaves are shown in Fig. 11). The first back-cross to *E. pseudokerneri* showed a corresponding approach to that species, but differed from it in its smaller flowers with more lilac colouring in them, showing the influence of *E. micrantha*. The only offspring of this back-cross was a single weak individual. Two F_2 plants were selfed and the progeny kept as separate lines in the following two generations. These two lines (numbered I and III) differed consistently in both the 1960 (F_3) and 1961 (F_4) generations. Line I approached *E. pseudokerneri* in having the branches more spreading and the flowers larger than in Line III, and in having relatively broad leaves at node 9, while it approached *E. micrantha* in its obtuse leaf teeth. Line III approached *E. pseudokerneri* in its more acute leaf teeth, but it was more like *E. micrantha* in its more erect branches, smaller flowers, and (in 1961) slightly more elongated leaves at node 9 (leaf 9 was not available in 1960). The leaf differences are shown in Fig. 12 and habit differences in Fig. 13. Some figures for flower size in this cross are given in Table 15. It will be seen that the range of the F_2 hybrid does not overlap with that of either parent in 1959. The range of the back-cross to *E. micrantha* only just overlaps that of *E. micrantha*, while that of the back-cross to *E. pseudokerneri* overlaps considerably that of *E. pseudokerneri*. The table shows that the larger the flowers the greater the range of variation; probably the larger flowers are

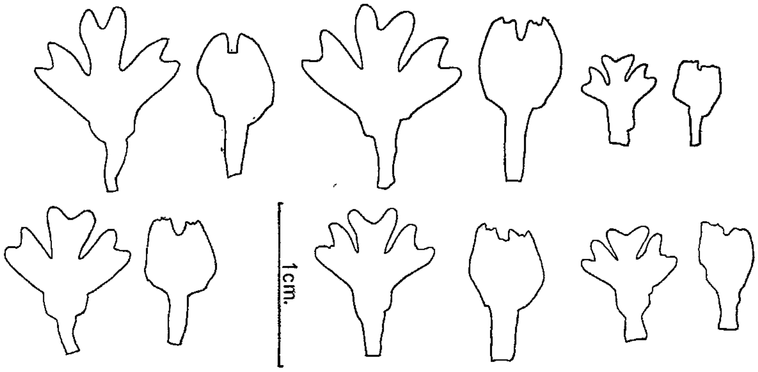

Fig. 10. Flowers of *Euphrasia*. Above, left to right, *E. pseudokerneri*, E609 (1959); the same (1956); *E. micrantha*, E185 (1956). Below, left to right, E609 × E185 back-crossed to E609 (1959); E609 × E185 (1958); E609 × E185 back-crossed to E185 (1959). × 2·2.

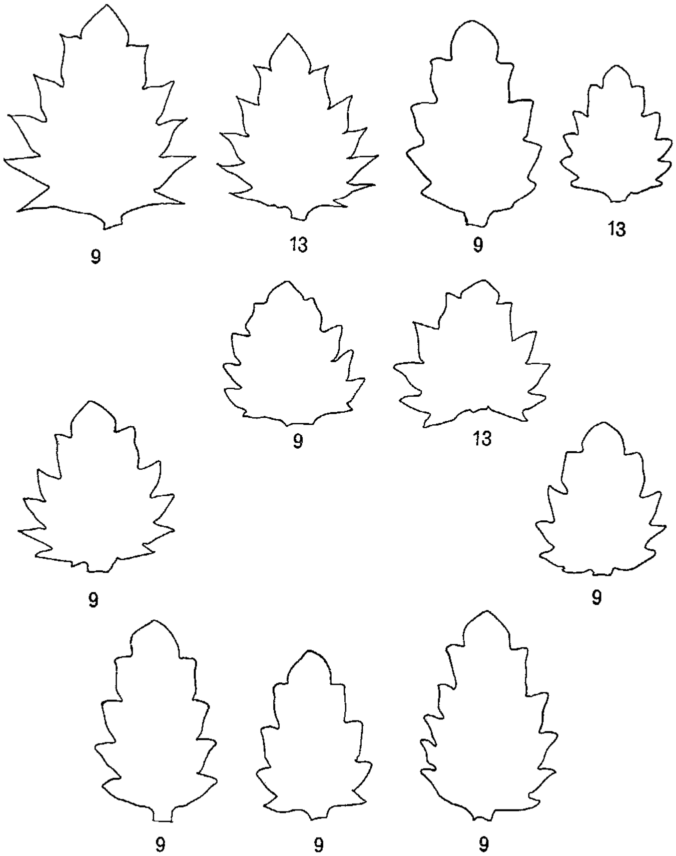

Fig. 11. Leaves of *Euphrasia*. Upper row, left, *E. pseudokerneri*, E609; right, *E. micrantha*, E185. Second row, E609 × E185, F_1. Third row, left, F_1 back-crossed to E609; right, F_1 back-crossed to E185. Lower row, right, selfed offspring of back-cross to E185; centre and left, second generation selfed offspring of back-cross to E185, two plants. Numbers indicate node from which leaves were taken. × 2, except lower left and centre which are × 3·6.

more influenced in size by external conditions (chiefly water supply and humidity). The large overlap between *E. pseudokerneri* and the back-cross to it is therefore probably caused by this greater variability of large flowers, and is thus an environmental effect.

The cross *E. salisburgensis* var. *hibernica* × *E. occidentalis* (E230 × E192) produced vigorous offspring (Fig. 14). These hybrids differed from *E. salisburgensis* var. *hibernica* in their greater size, stouter stems and branches, and coarser foliage; they were no less freely branched than the female parent, and in this way did not approach the sparingly branched *E. occidentalis*. This development of branches may have been a reflection of the vigorous growth of the plants, which in turn appeared to be connected with their very poor seed production. Both the leaves (Fig. 15) and the flowers were larger than those of the parents but were intermediate in shape. The leaves of all four hybrid plants had a few stalked glands, and had more eglandular hairs than in the female parent (in *E. occidentalis* both glandular hairs and eglandular bristles were numerous).

In the comparable cross between *E. salisburgensis* and *E. nemorosa* (E515 × E608) the hybrids were very sparsely branched, imitating the form of *E. salisburgensis* which had been used, and showing scarcely any approach in this to *E. nemorosa*, which is freely branched. In the single plant of the reciprocal of this cross (E608 × E515), branching was intermediate between that of the parents. Although the plants were not particularly vigorous, two were still flowering on 14 October, probably as a result of their failure to set any appreciable quantity of seed. The leaves (Fig. 15) and flowers were intermediate in shape between those of the parents.

The only progeny that could be obtained from the cross E515 × E608 were the result of open-pollination. Seed produced in this way yielded two plants in 1958, both with flowers much larger than, and very different in shape from, those of the F_1 or the two parent samples. It seems likely, therefore, that both plants were crosses between the F_1 and something else, in one case probably *E. pseudokerneri*, and in the other *E. pseudokerneri* or one of its hybrids. In characters other than shape and size of flower, these probable parents are comparatively close to *E. nemorosa*, and the cross with them was, therefore, virtually

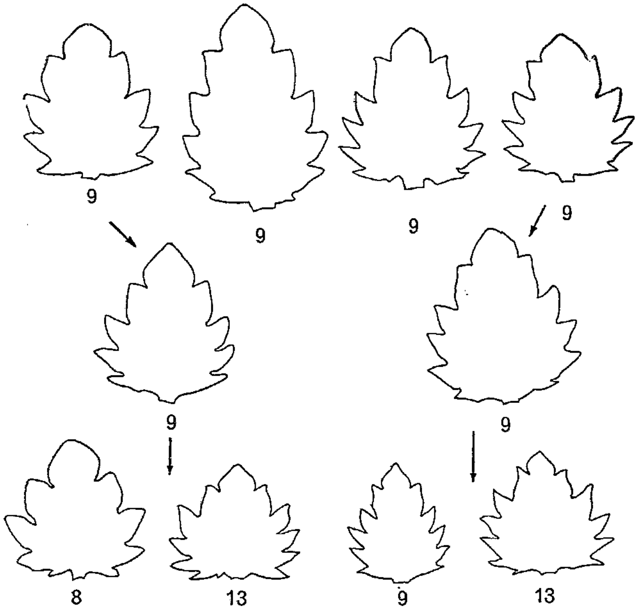

Fig. 12. Leaves of *Euphrasia*. Upper row, *E. pseudokerneri* × *E. micrantha*, E609 × E185, F_2 (1959), four plants; middle row, F_3 (1960), two plants; lower row, F_4 (1961), two plants. Arrows at left indicate Line I; at right Line III (the F_3 plant shown is a sibling of the actual parent of the F_4). × 2.

a back-cross. Because of this, the foliar characters of *E. salisburgensis* were practically lost in one plant and only weakly evident in another. The reciprocal cross (E608 × E515) was open-pollinated likewise, and gave rise to one offspring which also appeared to be the result of crossing with another plant.

The two back-cross plants: E515 × E608 × ?, were deliberately back-crossed to *E. nemorosa*, but with the possibility of selfing not excluded, and one of the three progeny

Fig. 13. Pressed specimens of *Euphrasia*. Above, left, *E. pseudokerneri*, E609 (1959); right, *E. micrantha*, E185 (1959); centre, second generation selfed offspring of back-cross of E609 × E185 to E185 (1961). Below, E609 × E185, F_4; left, Line I; right, Line III (1961). × ½.

(1959) showed a close approach to this species in its flowers, while the other two could have resulted from accidental selfing. The leaves of all three plants had finer teeth than those of *E. nemorosa*, a feature which probably owed something to the *E. salisburgensis* element in the plant's ancestry. The sparingly branched habit was evident in this series of crosses down as far as this generation and again in 1961, but not in 1960. From the plant with flowers like *E. nemorosa* two further generations were bred by selfing; among these the leaf teeth were on the whole more antrorse than in *E. nemorosa* and *E. pseudokerneri*, while one plant of the final (1961) generation had flowers remarkably like those of E515. Otherwise these plants showed the effects of *E. salisburgensis* in their constitution only by their marked, but declining, sterility. Another plant of this second (1959) back-cross generation was open-pollinated and one of its offspring appeared to be the result of a cross with the hybrid *E. pseudokerneri* × *E. micrantha*.

In Table 15 it was shown that the F_2 generation of *E. pseudokerneri* × *E. micrantha* did not overlap with the parents in flower size. Failure to obtain segregants corresponding with the parental types suggests that the inheritance of this character is controlled polygenically (that is, by polymeric or multiple genes, in the sense of Clausen and Hiesey (1958)). Multiple gene inheritance is suggested for most characters in all the crosses by the inter-

Fig. 14. Pressed specimens of *Euphrasia*. Left, *E. occidentalis* E192 (1953); right, *E. salisburgensis* var. *hibernica* E230 (1954); centre, E230 × E192, F_1 (1954). × ½.

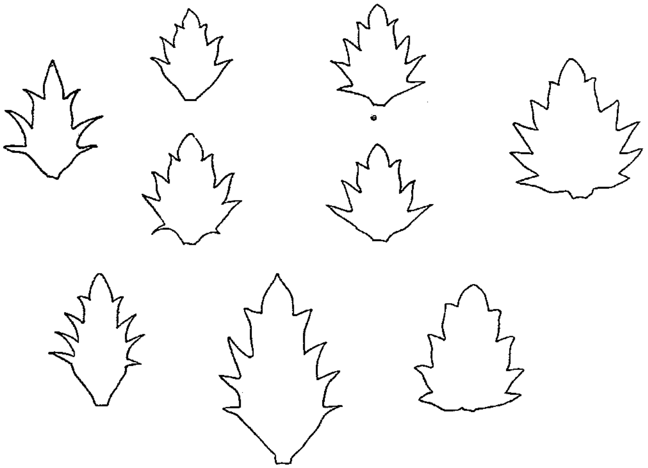

Fig. 15. Leaves of *Euphrasia*. Above, extreme left, *E. salisburgensis*, E515 (1956); extreme right, *E. nemorosa*, E608 (1956); centre, E515 × E608, F_1 (1957), four plants. All from node 13. Below, left, *E. salisburgensis* var. *hibernica* E230 (1954); right, *E. occidentalis*, E192 (1955); centre, E230 × E192, F_1 (1954). All from node 11. × 2.

mediate condition of the F_1 (indicating absence of dominant genes of large effect) and by the absence of extreme segregation in the F_2 and back-crosses. This can be seen to some extent in the various figures and tables dealing with hybrids, and it also applied to flower colour in the cross *E. pseudokerneri* × *E. micrantha*, the former having a mainly white ground colour, and the latter a purplish one. A notable exception was the straw-yellow corolla colour in *E. micrantha* × *E. confusa*, which appeared to be dominant in the F_1. There were indications that branching was controlled mainly by *E. salisburgensis* when this species was a parent.

It was found possible, in the course of five generations, to select different forms of the cross *E. pseudokerneri* × *E. micrantha* which bred reasonably true for different combinations of parental characters, and to obtain, by back-crossing, forms which showed chiefly the characters of the back-cross parent, somewhat modified. Rather uniform populations which appear to correspond to such different types can be found in nature, sometimes in situations giving direct evidence of hybridization and sometimes where there is no such evidence.

In order to amplify the picture of species relationships in *Euphrasia* an account of floral biology and natural hybridization will now be given.

FLORAL BIOLOGY

An account of the pollination mechanism of *Euphrasia* which is well supported by my own observations is that of Wettstein (1896) which is superbly illustrated. Wettstein grouped the flowers into three classes according to size and behaviour. In the species with large flowers, attaining 12–15 mm in length (exemplified by *E. rostkoviana*, *E. versicolor*, *E. picta* and *E. kerneri*) the individual flowers are at first female and later enter a male phase. When a flower of *E. rostkoviana* first opens, the stigma is held so that an insect seeking nectar will, if it is the right size, brush it with its back. Subsequently the corolla tube elongates and the anthers dehisce. An insect entering now strikes the prolongations of the anther cells, and shakes the pollen on to its back. If the insect then visits a flower at the female stage, it will cause pollination. The elongation of the corolla has led to the withdrawal of the stigma, so that the flower is functionally male. When a flower is in the second stage, there may be a flower in the first stage at the node above, so that self-pollination of the plant can occur. The large flowers are, however, primarily adapted to cross-pollination by insects.

The second type of flower is the middle-sized, exemplified by *E. stricta* and *E. pulchella*, in which the corolla reaches a length of 8–10 mm. In the half-opened flower, the stigma is in a position like that in the first stage of the large flowers, but the style is more curved. This short female phase lasts only until the flower is fully open, when the anthers dehisce. The flower is now functionally hermaphrodite. The style bends further bringing the stigma below the anthers. The stigma then withers and withdraws, while the anthers still produce pollen, so that the flower is functionally male.

In small-flowered species (*E. salisburgensis, E. micrantha* and *E. minima*) with flowers 4–7 mm long, the anthers have dehisced by the time the flower opens, so that the flower is initially hermaphrodite. The stigma lies immediately in front of or beneath the anthers, and self-pollination thus appears to occur regularly at the beginning of anthesis. However, the stigma soon withers, and the flower then enters a male phase.

Wettstein noted that the floral behaviour most strongly adapted to out-crossing was found in the largest flowers, while behaviour most strongly adapted to self-pollination was found in the smallest. Floral behaviour therefore corresponded with the conspicuousness of the flowers and their presumed attractiveness to insects. Since the basic morphology of the flowers is clearly adapted to insect pollination, Wettstein concluded that large flower size is primitive.

It was pointed out by Pugsley (1930) that in all Eyebrights the corolla grows during anthesis, and that this growth involves some elongation of the corolla tube. However, growth of the tube was considered by Pugsley to be disproportionately great in the large-flowered species, so that at maturity the corolla tube is exserted.

My observations have been made on *E. pseudokerneri, E. anglica, E. confusa, E. brevipila, E. nemorosa, E. micrantha* and *E. scottica* (this list is in order of decreasing corolla size, see Table 16). Detailed observations on floral development showed that this was subject to variation in the same species or even on the same plant.

TABLE 16. Length of corolla (mm), measured along the upper lip, of some samples of *Euphrasia*.

	Initial	*Final*
E. pseudokerneri, E42	6·5–7·0	8·5–9·5
E. anglica, E76	6·5–8·0	7·5–9·0
E. anglica, E71	5·0–7·0	7·0–8·0
E. confusa, E209	—	7·0–8·5
E. brevipila, E81	—	6·5–8·5
E. nemorosa, E196	—	6·0–7·0
E. micrantha, E185	—	5·5–6·0
E. scottica, E120	—	4·5–6·5

In *E. pseudokerneri* it was found that when the flower opens the stigma is curved through approximately a right angle but held well clear of the anthers, so that even if it is slightly below the level of the anther openings, it is out of the line of fall of pollen. If pollination does not take place the style remains in about the same position, or bends more to bring the stigma within reach of pollen falling from the anterior anther-loculi. After pollination the style straightens out and the stigma is usually withdrawn into the space between the anthers and the upper lip of the corolla. The withdrawal of the stigma is probably caused by the elongation of the corolla tube, but towards the end of anthesis the base of the style may wither, and as this probably results in shrinkage it may contribute to withdrawal of the stigma. Flowers usually open in the morning in *Euphrasia*, and in *E. pseudokerneri* the anthers are usually then undehisced; however, at least the anterior loculi usually dehisce later in the first day. The anthers are just concealed by the upper lip of the corolla, when it is viewed from the side, but later they protrude slightly. The flowers normally last for four days but some lasted for six; the corolla continued to expand during most of this period (not merely until the anthers dehisce as stated for all species by Pugsley (1930)).

Three samples of *E. pseudokerneri*, a large-flowered one of *E. anglica*, and one of *E. brevipila*, all behaved in much the same way. They seemed to fall between Wettstein's large-flowered and medium-flowered types in behaviour, since the stigma very occasionally came down to where the pollen could fall onto it, and sometimes (usually in *E. brevipila*) the anthers had already dehisced when the flower opened. A second, smaller-flowered, population of *E. anglica*, together with *E. confusa* and *E. nemorosa*, was similar to Wettstein's medium-sized type, for the style curved strongly after the beginning of anthesis so that the stigma was below the anthers or touching their lower front edge. The anthers dehisce just before the flowers open. In *E. scottica* the stigmas were not found below the anthers, but had doubtless been in this position because there was pollen on them when the flowers opened. It was found in *E. micrantha* also that dehiscence of the anthers took place well before the flowers opened. These last two species were therefore in Wettstein's third class. Occasionally, anther-dehiscence took place in *E. nemorosa* and *E. micrantha* long before, instead of immediately before, the flowers opened. This may have been caused by water shortage or hot weather.

In addition to Wettstein's and Pugsley's accounts of floral development in *Euphrasia*, a full account was given by Müller (1883) who mentioned that the pollen is dry and powdery, and that it falls from all the anthers when the spines, which are on the back anthers, are touched. His account of the style elongating after the flower opens in *E. micrantha* is probably mistaken. Schulz (quoted by Knuth 1909) recognized seven flower-forms, but some of these are probably based on the variability of behaviour that I have observed. While some authors state that the largest-flowered Eyebrights cannot be selfed, others suggest that they are self-pollinated to some extent. On one occasion I knocked a young flower of *E. pseudokerneri*, and this brought pollen onto the stigma, so disturbances other than by insects may cause self-pollination, even if the stigma appears to be out of the line of free fall of the pollen.

The ground colour of the corolla is either white or lilac to purple. The lilac colouring is usually slightly stronger in the upper lip and it intensifies slightly with age. Flowers with a white lower lip may thus have a pale lilac upper lip, and flowers which are initially white may become pale lilac. Lilac colouration develops more strongly in cool weather than in warm. Both lips of the corolla usually have some of the longitudinal veins coloured dark violet, forming guide-marks converging towards the throat; the extent and intensity of this coloured veining varies. Near the base of the lower lip is a yellow spot, and there is another just inside the tube on the lower side. The intensity of these marks is also variable. Some pollen always falls from the anthers and most of it usually falls onto the yellow spots. The nectary is at the base of the ovary on the lower side. It is often difficult to find nectar in the flowers, but it sometimes collects in a drop some distance away from the base of the corolla tube, presumably owing to some degree of resistance to wetting by the base of the tube. According to Schulz (see Knuth 1909), the nectary is well-developed in the larger-flowered species and less well-developed or absent in the smaller-flowered species.

The main insect visitors to *Euphrasia* flowers are hover-flies (Diptera: Syrphidae) and bees (Hymenoptera: Apidae)—chiefly *Bombus* (bumble-bees). This is true both in Europe (Daumann 1932, Knuth 1909, Müller 1883, Wettstein 1896) and in Britain (Drabble 1927, Scott-Elliot 1896, Willis & Burkill 1903). Diptera and Hymenoptera of other familes have also been recorded, as well as Lepidoptera (Drabble, Knuth, Scott-Elliot, *l.c.*).

My observations on the behaviour of insects visiting the flowers have been made chiefly in glasshouses, where insect visits are quite frequent. However, the greatest insect activity at the flowers of *Euphrasia* that I have seen occurred in 1953 when I had a plot in the garden at Leicester containing over three hundred extremely luxuriant and profusely flowering specimens of *Euphrasia*. This patch of plants became a considerable attraction for insects, which could be seen continually active at the flowers in warm weather. Here eight species of Syrphidae were taken and species of *Bombus*, *Apis* and *Halictus* (all Apidae) were seen visiting the flowers. I have only rarely seen *Euphrasia* visited by insects in nature, having taken a total of five specimens, and seen about the same number again which I have not caught. The visitors that were identified were *Bombus*, *Halictus*, *Empis* (Diptera: Empididae) and Syrphidae. The flowers visited ranged from the small ones of *E. micrantha* to the large ones of *E. pseudokerneri*. The bee visitors appear to probe for nectar, and sometimes pollen collects on the thorax. Females doubtless gather this into their pollen carrying apparatus, but the males of solitary bees probably have no use for the pollen. Syrphidae probe for nectar, and in doing so put their head and thorax into the throat of the corolla. When feeding on pollen they straddle the lower lip and their proboscis takes up pollen that has fallen into the throat of the corolla from the anthers and any loose pollen from the stigma and anthers. Thus, the insect's head probably comes into frequent contact with the anthers and stigma. It seems possible that the function of the yellow spots is to heighten the visual effect of the patch of pollen which is usually present. Judging from the behaviour of Syrphid visitors, the pollen is at least as much of an attraction to them as the nectar.

THE OCCURRENCE OF HYBRIDS AS EVIDENCE OF CROSS-POLLINATION

In addition to finding wild hybrids, I have obtained spontaneous hybrids from seeds collected from cultivated plants. Two instances are of interest.

It has already been described how the flowers of *Euphrasia* (mainly *E. nemorosa*) grown in the garden at Leicester in 1953 became an attraction to insects. Six populations of *Euphrasia* were grown, well intermingled. Three populations were closely similar, and three others all easily distinguished by eye from the rest and from each other. The one which was most distinct was E151A, and it therefore appeared that it would be easy to pick out hybrids in the progeny of this form. Seed was collected from most of the plants of this population. At the time of collecting, its flowers were greatly outnumbered by those of other populations. The following year the progeny were grown at Cambridge by the same method as the parents; 79 bituminized paper pots, each with one *Euphrasia*, were planted out. The final score was 14 plants of the maternal type and 42 hybrids. That is, 75 per cent. of the survivors were hybrids. The maternal type, which showed heavy mortality and late establishment in 1953, showed late establishment and lack of vigour in 1954, and probably suffered heavier mortality than the hybrids as a consequence. The proportion of hybrids in the seed-sample was, therefore, doubtless less than 75 per cent. Eighty-seven seedlings, including replacements, were planted out, so that if all those that died were of the maternal type the proportion of hybrids was 48 per cent. Therefore, the true figure was not less than 48 per cent. and not more than 75 per cent.

However, the following year about 200 more seeds of the original sample germinated. Eighty-two seedlings were still alive on 4 June 1955, and 30 were then potted up and grown in the greenhouse. In this culture, nine plants of the maternal type and only four hybrids lived long enough to be identified. The ratio of the two types among survivors was thus reversed. If, as seems likely even from this small sample, the ratio of maternal to hybrid types was really changed, a possible explanation is that hybrid types germinated more readily than the maternal in the first year. While this experiment cannot give a reliable estimate of the proportion of hybrids in the seed produced by the plants of E151A grown in 1953, it does show that there was some hybridization between individuals, and that insect-pollination was quite effective. *E. nemorosa* is a fairly small-flowered species, and it probably generally behaves like Wettstein's intermediate flower type.

In the second instance, a quantity of open-pollinated seed of the small-flowered *E. micrantha* (E185A), collected in 1953, was sown on 17 February 1954. Apparently because of the lateness of the sowing, only two seeds germinated in that year, but both plants were grown to maturity, and both proved to be hybrids of *E. micrantha*. In 1955 about 49 more seeds germinated, and 18 of these were potted up. Only four grew well enough to be identified and none was a hybrid. This result again suggests that the hybrid types may germinate more readily than the maternal. In view of the floral behaviour of *E. micrantha*, it was rather surprising to find two hybrids in this fairly small seed-sample. Presumably, pollen brought to a flower just after it has opened has a fair chance of effecting fertilization, even when the anthers have already dehisced.

Wild hybrids may belong to the F_1 generation, to the first back-cross generation, or to succeeding generations. The occurrence of wild hybrids is thus less informative than that of spontaneous hybrids in cultivation, because only in the latter can the F_1 generation be recognized with certainty; the occurrence of wild hybrids does, however, show that cross-fertilization occurs in nature. In a survey of my herbarium collection covering the years 1951–1958, I found that I had met with 11 dense populations of hybrids between species alike in chromosome number extending over considerable areas, and 16 well defined colonies of relatively small size. In addition, I found 22 *Euphrasia* populations containing more than one species of the same chromosome number and containing a very few hybrids. (In Cornwall I have several times found plants apparently intermediate between *E. nemorosa* and *E. confusa* unaccompanied by typical plants of either of these species; these have been excluded from the totals). Ecological conditions appear to have a very marked effect on the occurrence of hybrids. For example, although *E. salisburgensis* produces highly sterile progeny when crossed with tetraploid species belonging to Subsection *Ciliatae*, a large gathering of a hybrid, apparently fertile, between *E. salisburgensis* var. *hibernica* and *E. nemorosa*, was obtained from Lough Bunny, County Clare, by Professor Tutin in 1951. One can only suppose that the hybrids are highly favoured ecologically in this locality and that they eventually become established with a reasonable degree of fertility. Similar assumptions have been made with regard to forms of diploid species showing introgression from tetraploids (Yeo 1956). A contrary example concerns *E. micrantha* and *E. confusa*. These two were growing in contact on a moor north of Minions, E. Cornwall, where there was a vegetational pattern consisting of areas of *Erica cinerea*, *Calluna vulgaris* and *Ulex gallii*, divided up by grassy areas; *E. confusa* grew in the grassy areas, and *E. micrantha* grew around the *Erica* and *Calluna* plants; no hybrids could be found here, but some were found a little way away growing with the parents on the top of a disused railway embankment; the hybrids formed a small group of uniform plants where the vegetation ended in stony ground. The scarcity of hybrids here appears to have been caused by habitat conditions.

The regular occurrence of wild hybrids demonstrates that cross-pollination is normally possible for Euphrasias in Great Britain. It seems reasonable to infer, therefore, that there is in nature an appreciable gene-flow over short distances in most *Euphrasia* populations, including those of small-flowered species.

DISCUSSION

It is well known that the genus *Euphrasia*, at least in Europe, is one of considerable taxonomic difficulty (see, for example, Pugsley 1930, Joergensen 1919). It consists of numerous poorly defined microspecies, many of which interbreed freely where they meet. These microspecies may be very wide-ranging (e.g. *E. tatarica* and *E. hirtella* range from the Pyrenees to eastern Asia), of moderate range (e.g. *E. rostkoviana* and *E. micrantha* of central and northern Europe, including the British Isles) or of very restricted range, down to areas as small as an English county (e.g. *E. cambrica*, from Caernarvonshire). Taxonomic difficulty is increased by the occurrence of aberrant forms of particular species (which could be the result of past introgression), forms assignable to hybrid parentage, though at present unaccompanied by the parent species, and occasional very local but distinctive forms which are most probably hybrid segregants but are difficult to assign to any stated parentage, and too restricted to be worth naming.

The results given in the present paper show that there is normal sexual reproduction in *Euphrasia* and that sterility barriers are formed by polyploidy and by homoploid hybrid sterility. However, there are only two levels of 'ploidy' and gene exchange is possible even between these (Yeo 1956), while many homoploid species are separated by no sterility barrier. More often than not, therefore, any two species are highly interfertile. The amount of out-crossing to which the different species are adapted is variable, but out-crossing is sufficiently common to make hybrids frequent, even between small-flowered species. On the other hand, the majority of seeds probably result from self-fertilization, at least in all but the largest-flowered species, so that the products of hybridization can probably quickly acquire uniformity. I have on a number of occasions seen quite uniform gatherings in which there was strong evidence of hybrid origin, and my experimental evidence shows that five generations is enough to produce two lines of hybrids derived from one cross, which differ much less among themselves than between each other.

All the microspecies have definite ecological and geographical characteristics, and many show obvious adaptations to major habitat differences (e.g. hay-meadows *versus* sheep grazings). Many species are separated geographically, but in most parts of Europe where the genus occurs at all, more than one species is to be found.

The factors of internal sterility barriers, geographical isolation, and possibly floral biology, contribute to the maintenance of the distinctness of species, but where they do not operate, and hybridization occurs, other factors must come into play, for the species can be found again and again in approximately the same form over most of their range. These other factors must be the selective influence of the habitat and genetic linkage. Anderson (1949) showed that when characters are polygenically controlled there is a strong tendency for the characters of one or other parent to remain associated in the F_2 hybrid and later generations (a phenomenon called genetic cohesion), and that there is a tendency for hybrids of later generations to comprise increasing proportions of individuals resembling one or other parent. These tendencies would be enormously reinforced by back-crossing.

It is necessary to consider the impact of present knowledge of the group on its taxonomic treatment. It seems to me that this knowledge requires either that the taxa recognized currently as species should remain at that rank, or that they should be reduced to the level of subspecies. From a practical point of view, it seems much preferable to continue treating them as species. Not all species are of the same standing, and it would seem that the *Euphrasiae* can justifiably be retained in this category, though they may represent its lowest limit.

In genecological terms, the *Euphrasia* species would be ranked as ecotypes in the sense of Clausen and his school, but higher than the ecotypes of Turesson. There is no doubt that the commoner and more successful *Euphrasia* species include ecotypic variants. It seems inevitable that any species which consists of numerous more or less disconnected local populations (which is the case in the *Euphrasiae*) will include every possible degree and type of ecotypic differentiation.

It will be seen from the appendix that the diploid species (Series *Hirtellae* only) are fewer in number than the tetraploids and are less wide-ranging, their northern limit in Britain being Argyll and Perthshire. In addition, diploids in general are much less abundant than tetraploids; where one finds diploids one almost invariably finds tetraploids also, while the reverse is very often *not* the case. *Euphrasia* thus conforms to the widespread pattern in vascular plants, in which the diploids are generally less diversified and narrower in their tolerance than polyploids.

APPENDIX: THE ECOLOGY OF *Euphrasia*

The following is a brief summary of the ecology and distribution of the more important British *Euphrasiae*, arranged in systematic order. The habit gives an indication of the height of herbage to which the species is adapted.

Series Latifoliae

1. *scottica*. Wet flushes in mountain areas in N. and W.; habit tall.
2. *micrantha*. Callunetum, absent E. Anglia, probably extinct S.E. England; habit tall to dwarf.
3. *frigida*. Alpine grassland, streams, ledges; habit dwarf to medium.
4. *foulaensis*. Maritime, northern; habit dwarf.
5. *campbelliae*. Maritime, Outer Hebrides; habit dwarf.

Series Nemorosae

6. *rotundifolia* and *marshallii*. As 4.
7. *curta*. Open habitats (dunes, rock outcrops, river shingle), middle-northern and north-western; habit dwarf to short.
8. *cambrica*. Alpine, N. Wales only; habit dwarf.
9. *occidentalis*. Maritime, southern to middle-northern; habit dwarf to short.
10. *nemorosa*. Roadsides, pastures, woods, dunes, southern, maritime in the north; habit tall to short.
11. *confusa*. Pastures, usually closely grazed, coastal to montane, mainly western and northern; habit dwarf to medium.
12. *pseudokerneri*. Grazed chalk and oolitic grassland, south-eastern; habit short to medium.

Series Brevipilae

13. *borealis* (vars. *zetlandica* Pugsl. and *speciosa* Pugsl. only). Pastures, meadows (?), Orkney, Shetland; habit medium to tall.
14. *brevipila*. Meadows, pastures, mainly western and (especially) northern; habit tall to dwarf.

Series Hirtellae

15. *rostkoviana*. Meadows, pastures, western and middle-northern; habit tall to medium.
16. *montana*. Meadows, middle-northern; habit tall.
17. *vigursii*. *Ulex* heaths, south-western; habit tall to medium.
18. *anglica*. Pastures, often closely grazed, England (except the north), Wales, Ireland; habit short to dwarf, sometimes medium.
19. *rivularis*. Alpine rivulets and flushes, middle-northern; habit dwarf.

Subsection Augustifoliae

20. *salisburgensis*. Grazed turf, lowland, W. Ireland; habit dwarf.

Nos. 4, 5, 6, 8, 11, 12, 13, 17, 18 and 19 are endemic to the British Isles, or nearly so. It is quite common for two species of *Euphrasia* to grow in mixed or closely adjoining populations. The wide-ranging *E. nemorosa* may easily come into contact with as many as nine other species, while more restricted species, such as *E. pseudokerneri* and *E. foulaensis*, may be in contact with three or four others.

Note: An interesting feature of the British *Euphrasiae* is the occurrence of two widespread endemics specially adapted to grazing (*E. confusa* and *E. anglica*), which do not seem to have their counterparts on the Continent of Europe. This is probably related to the prevalence of all-the-year-round sheep-grazing and the strength of rabbit pressure, both factors of very recent origin.

ORIGIN OF MATERIAL

Unless otherwise stated all *Euphrasia* plants grown are from the British Isles. Herbarium specimens of the original gatherings, if they exist, are to be found in my own collection, or in the Cambridge University Herbarium (CGE). Many duplicates are at the University of Leicester. All cultivated specimens are in my own herbarium.

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