EXPERIMENTAL TAXONOMY ON THE GENUS EUPHRASIA L.

(SCROPHULARIACEAE - RHINANTHOOIDEAE)

BY

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INTRODUCTION

Euphrasias are found throughout the British Isles, and are especially abundant in hilly districts. They are usually confined to more or less natural grassland, and to habitats where the grass tends to be short or thin. All the British forms are annuals, and the flowering period is from June to October, though most species flower only during part of this period.

Morphologically the plants are rather simple. The leaves are produced in pairs, and are usually opposite, although the upper ones may be alternate. The first pair of leaves has one obtuse tooth on either side. The number of teeth increases at higher nodes, and they become more acute. There is also a transition in leaf outline from the base to the apex of the plant. Branch initials are produced in the cotyledonary and lower foliar axils. How many are produced, how many grow, and how soon they start to grow depends on the species, and on how well-nourished the individual plant is. Nourishment also affects the degree to which the branches themselves produce branches. On the upper part of the main axis and branches there is a transition from branch production to flower production, but the first flower initials frequently fail to develop. The flower is described fully later; all the British species are alike in the lobing and markings of the corolla, variation being confined to size of corolla, shape of lobes
and colour. After anthesis the bilocular capsule enlarges to approximately the length of the calyx. The two loculi open at the top when ripe and split down the side for some distance. The seeds are fusiform and furrowed, and about 1.5 to 3 mm. long. In all probability, the uppermost seeds in a capsule drop to the ground soon after dehiscence; most of the rest are probably flung out when the wind blows, but a few remain at the bottom of the capsule and are probably only released when it rots in winter.

The history of the taxonomic treatment of Euphrasia is told by Pugsley in his "Revision of the British Euphrasiae" (1930) and his "Enumeration of the Species of Euphrasia L., Sect. Semicalcaratae Benth." (1936). The foundation of the modern view of the genus was laid by Wettstein in his "Monographie der Gattung Euphrasia" (1896), in which 87 species were recognized. The species concept in the genus has changed little since 1896, but many new species have been described. Wettstein's rather simple system of classification was modified by Joergensen in his very detailed account of Norwegian Euphrasias (1919), and again by Pugsley in the works just mentioned. Pugsley's "Enumeration" listed 96 species, although it omitted many taxa with which he was not properly acquainted, and covered only that part of the genus represented by Wettstein's first 54 species. Many recently described or new species are included in "Flora U.R.R.S."

In the southern hemisphere, the genus Euphrasia occurs
in South America, Australia, New Zealand, Tasmania, New Guinea, and Celebes. The species in these areas include shrubby perennial as well as annual plants. Perennial species occur in the northern hemisphere in Borneo, the Phillipines, Formosa and the Azores. In Japan there are a number of annual species, which simulate other northern annual forms in habit but are fairly clearly set off from them in leaf and flower characters. Pugsley placed them in Section Semicalcaratae and in two Subsections, Japonicae and Alpicoles. The rest of the northern Euphrasias belong to two Subsections of the Section Semicalcaratae which are both represented in the British Isles. One is Subsection Angustifolii, in which Pugsley (1936) recognizes 12 species. This Subsection is confined to Europe and Turkey, and is represented in the British Isles by *E. salisburgen-sis* var. *hibernica*. 75 other species listed by Pugsley belong to the other Subsection, Ciliatae. Subsection Ciliatae occurs in Alaska, Canada, N.E. United States, Greenland, Morocco, throughout Europe, and across Asia to Japan and Kamchatka, extending south to the Himalayas. This Subsection is the most widely distributed in the northern hemisphere, and the richest in species. It is divided into eight Series, of which four, Latifolii, Minutiflorae, Pectinatae, and probably Nemorosae, are represented by native species both in Europe and eastern North America. Minutiflorae is primarily an American group with a European representative, *E. botttnica*, confined to
the Finnish and Swedish shores of the Baltic; *E. frigida* (Series Latifoliae) is a species common to Europe and North America; the European *E. curta* (Series Nemorosae) is probably also native in North America; and the European and Asian Series Pectinatae is represented in North America by *E. hudsoniana*. In addition, three or four European species have been introduced into eastern North America. Connecting western North America and eastern Asia is *E. mollis* (Series Latifoliae) which is common to both regions; another western North American species, *E. subarctica* of the Eurasian Series Hirtellae, also seems to connect America with eastern Asia.

Some *Euphrasia* species are very wide-ranging, *E. hirtella* extending from West Europe to East Asia, and *E. tatarica* from South-east Europe to East Asia. Others, such as *E. frigida*, *E. rostkoviana* and *E. hudsoniana*, are also wide-ranging within continents. Most areas where Euphrasias occur are inhabited by both wide-ranging species and species with a more or less restricted distribution, and it is probably the case that the more favourable the area to Euphrasias the more species will be found there. The British Isles and the Central European Alps are two areas particularly rich in *Euphrasia* species. Pugsley finally recognized 24 species in the British Isles; the treatment of Warburg (1952) follows Pugsley very closely, and has been used in preparing the following list of British Euphrasias, classified according to distribution.
Endemic to British Isles; occurring in not more than four vice-counties

- rhumica
- eurycarpa
- campbelliae

Endemic to British Isles, or nearly so; more widespread

- foulaensis (also Faroes)
- marshallii
- occidentalis (also Brittany)

British Isles and more northern countries

- scotica (also Scandinavia)
- frigida (also W. Siberia and westwards to Hudson Bay)
- rotundifolia (also Iceland)

British Isles and rest of Europe

- micrantha
- nemorosa
- brevipila

British Isles, rest of Europe, and Asia

- hirtella

The taxonomy of *Euphrasia* is particularly difficult, because the species are distinguished by smaller differences than is usually the case and, although several differences between a species and its nearest relatives may be described, these differences are usually in characters capable of slowing a good deal of fluctuation. The most important taxonomic characters in *Euphrasia* are internode length in
relation to leaf length, the node at which flowering begins, the number, length and direction of the branches, the indumentum and shape of leaves and calyx, size of flower, and shape of calyx and capsule. Additional difficulty is caused by the fact that Euphrasias are annuals and semiparasitic; this means that great variation occurs in vigour, which influences many of the distinguishing characters. Apart from this, there is much local variation, and hybridization occurs freely. From time to time, one also meets with populations which cannot be referred to any of the recognized species, nor plausibly ascribed to hybrid origin.

In spite of the difficulties, the fact that about 95% of gatherings can be named and that the different forms occupy distinct habitats and can be recognized over more or less considerable geographical areas (often interrupted by areas of unsuitable habitats) encourages one to apply orthodox taxonomy to the recognizable forms and to treat them as species. One must not, however, insist on having a name for every specimen, and it must be recognized that the species of *Euphrasia* show closer relationships with each other than is usual in the less critical groups.

The work now to be described is an investigation into the background of this taxonomic situation in the genus, in regard to parasitism, nuclear cytology, breeding behaviour, and local variation. The Euphrasias employed were almost all British species, although some of the seed samples came
from other parts of Europe.
ACCOUNT OF PREVIOUS WORK ON THE DEVELOPMENT OF EUPHRASIA

I. Germination

All the investigators quoted below on the subject of germination have found that germination is independent of the presence of roots of other plants. In addition, Heinricher (1898 b) obtained the germination of solitary seeds in pots containing two or three seeds each.

Koch (1891) sowed seed of *Euphrasia officinalis* L. (an aggregate species; the species most likely to have been employed are *E. stricta* Host, and *E. rostkoviana* Hayne) in pots in the autumn, and obtained free germination the following spring. It is not stated whether the pots were in the open or not.

Wettstein (1896) described some germination experiments in his monograph on *Euphrasia*. He sowed seed in troughs in the open (in some of which seed of other plants had been sown previously, in others of which the soil was root-free) and on damp blotting paper between the windows of a room at 4-10°C. The species sown in the open were:

- *E. rostkoviana* from three different localities
- *E. hirtella* Jord.
- *E. numila* Kerner
- *E. salisburgensia* Funck
- *E. minima* Jacq. (two varieties).

Only *E. rostkoviana* and *E. hirtella* were sown on blotting paper. Seed of all these species, collected in 1893 and
sown on 10 October 1893, germinated in March 1894, germination indoors being simultaneous with, or up to eleven days earlier than, that outdoors. Seed of *E. minima* and *E. rostkoviana*, sown under the same conditions on 25 January 1894, germinated five to twelve days later than the corresponding October sowings. Seed of *E. rostkoviana* from 1893, sown outdoors on 1 April 1894, failed to germinate; sown indoors on blotting paper on 3 April 1894, 3 seeds out of 70 germinated on 15 April 1894. Seed of *E. rostkoviana* collected in 1892 and sown on 10 October 1893 failed to germinate.

A percentage germination of 70 (42 out of 60 seeds) is recorded by Wettstein (1897) for *E. rostkoviana* sown in October.

Wettstein concluded that the time of germination was independent of the time of sowing; and that, if seed does not germinate during the spring following its formation, it loses its viability about the time when it would normally be germinating. He later stated, however, (1898) that the pots in which no germination had taken place were left only until the following October.

However, Heinricher (1898 a) demonstrated a different state of affairs in regard to germination time. Using *E. stricta* seed of the 1895 crop sown in pots in 1896 on 27 February, 28 March, 16 April, 22 May and 23 June, he showed that the two earliest sowings gave quite good germination in March and April respectively, the next two
poor germination in May and June respectively, and the last
gave no germination in 1896 but good germination at the
beginning of March 1897. In addition, a thick sowing of
1894 seed on 27 February 1896 gave 1 seedling in March
1896, and a sowing of 1895 seed on 24 January 1897 gave 36
seedlings by 3 March 1897. He thus demonstrated a restric-
tion of germination to the spring, and the retention of
viability into the second spring after formation, if
germination cannot take place in the first. It was not
stated where the pots were kept. Other results for
E. stricta are (Heinricher, 1898 a): of some sown in a pot
in a warm house on 21 January 1897, 1 seedling germinated
on 4 February; sowings in pots on 27 February 1896 had
started germinating by 17 March 1896; of many seeds sown in
a pot on 28 March 1896, 4 had germinated by 16 April, 20 by
2 May, and 36 by 15 May 1896.

E. rostkoviana, when sown in a pot on 30 December 1896
and kept in the window of the Botanical Institute, germi-
nated early in February 1897 (Heinricher, 1898 b). Three
or four seeds of this species sown in a pot on 27 February
1896 produced two seedlings on 22 March 1896 (Heinricher,
1898 a). Two March sowings are recorded for this species,
one on 4 March 1898 in which ten seedlings appeared between
24 March and 4 May 1898, and one on 2 March 1898 in which,
out of 20-30 seeds, six germinated in 1898 and "all the
rest" in 1899 (Heinricher, 1898 b).

E. salisburgensis was sown in open ground on 30
October 1896, and had germinated abundantly by 15 April 1897.

_E. minima_, sown in open ground on 4 November 1896, had started germination by 15 April 1897. A pot of _E. minima_ seed, sown at the same time and kept in the window of the Botanical Institute, had produced two seedlings by 21 February, and 27 by 7 April 1897 (Heinricher, 1898 b).

These results show earlier germination indoors than out, though in the case of _E. stricta_ it was apparently poor indoors; they also show that seed of _E. rostkoviana_ can, like that of _E. stricta_, germinate in the second spring after its formation. The time between sowing and germination is two to four weeks, unless the sowing takes place in the autumn or early winter, in which case germination is delayed until February or March. In Heinricher's experiments germination was confined to the period February to May.

Neidhart (1947) sowed seed of _E. rostkoviana_ collected in the years 1934 and 1936-38) in the winter of 1938-39, and in the spring of 1939. Samples of 100 seeds were sown on damp blotting paper in petri-dishes. They were subjected to various conditions of light and temperature. Indoor temperatures up to 30°C. and outdoor temperatures ranging from -5°C. to +5°C. were used. Varying alternations of high and low temperatures were employed also. Only the seed of the 1938 crop germinated, and about 20% germinated in all samples except that at a constant
temperature of 30°C., in which 10% germinated. A test using one seed per dish gave germination at the same time as the others.

Germination was said to take five to seven days; this is very quick, but germination would be visible sooner in seeds on blotting paper than in seeds in the soil; however, it seems extraordinarily quick for the sample outdoors in temperatures ranging from -5°C. to +5°C.

Seed samples sown for cultivation in March, with 1, 2, 5, or 100 seeds per pot of soil, germinated in 15-20 days, with percentages up to 40.

It will be noted that Neidhart failed to get seed to germinate except in the season after it was formed.

II. Other Aspects of Growth
in Nature and in Cultivation

Discovery of Parasitism in Rhinanthoideae.

It was discovered by Decaisne (1847) that members of the Rhinanthoideae formed haustoria on the roots of other plants.

That the group might be parasitic was suggested to him by the fact that he could not cultivate Melampyrum arvense, Euphrasieae, or Pediculaires by transplantation, nor M. arvense from seed. He then found the roots of Alectrolophus (Rhinanthus), Melampyrum, and Odontites, with haustoria attached to the roots of "Gramineae, of shrubs,
and even of trees". (Melampyrum is a parasite of shrubs and trees). It seemed to Decaisne also that this discovery accounted for the accusation of agriculturists that Rhinanthoideae, etc. exercise an injurious action on hay and cereals.

Decaisne's observations were reported in the Gardeners' Chronicle in 1847, and this led to a letter from Lawson (1847) in Scotland, who reported failure to find haustoria in Euphrasia, and mentioned digging up a plant in the middle of a cart track far removed from any other plants which was fairly certainly not parasitic. Another letter, from Henslow (1847), said he had, without difficulty, found haustoria in E. officinalis and E. odontites (Odontites verna) by digging up the plants and surrounding soil and soaking them thoroughly before removing the soil by gentle agitation and with a glue brush. The attachments were with grass roots. Henslow supposed that isolated Euphrasia plants he had found had destroyed their host plants.

Work of Ludwig Koch.

Koch studied Rhinanthus, Odontites and Euphrasia. His first paper dealt with Rhinanthus, and the second (1891) with Euphrasia officinalis L. (see p. 1). Seedlings germinated in pots grew much as wild ones do, if the pots contained old tufts of grass or grass seed sown at the same time as the Euphrasia seed (i.e. autumn). In Rhinanthus
minor, Koch had found that simultaneous sowing gave retarded growth. He attributed this to the more rapid growth and earlier maturity of Rhinanthus placing a big demand on the host plants while they were still relatively young. Koch also sowed grass seed in pots with Euphrasia seedlings 3cm. tall. (This figure may be an error; it seems too high in view of results to be described later). The effect of this was similar to that of simultaneous sowing with Rhinanthus, but worse, the Euphrasias dying off after three to four weeks. Euphrasias with no host plant grew about as weakly as those with host seed sown after germination; if the seedlings were thinly and evenly distributed, they appeared starved and died off from mid-June onwards; in thick, uneven distributions some plants grew faster than the rest and were found to be attached to their neighbours by haustoria. These plants died if surrounded by few others, but if surrounded by enough others they occasionally flowered. They were prevented from fruiting by being dug up for investigation of the roots.

Koch always found that haustoria only seized living roots, and they only parasitized the youngest or thinnest host roots. In Melampyrum, as opposed to Euphrasia, the haustoria attach themselves to dead organic matter. Saprophytism occurs in Euphrasia and Rhinanthus only after the end of the parasitic activity of the haustoria, when the affected host roots have died.

Koch concluded that parasitism in Euphrasia and
Rhinanthus was essential, not facultative nor merely incidental. Since Euphrasias have plenty of green foliage, he supposed that parasitism must especially supply raw material for protein (presumably nitrogen) and also (other) inorganic salts. The cultures indicated that direct root activity (i.e. non-parasitic activity) plays only a subsidiary part, or none, in the absorption of nutrients.

Koch investigated in detail the anatomy of the haustorium. Characteristic of Euphrasia are the small size and slight growth in length of the haustoria, the simple tracheal strand (formed from one row of cells), and the clasping structures round the edge of the contact surface of the haustoria. Root hairs are more or less isolated. The attacked host root becomes internally disorganized, and the attack becomes saprophytic. About this stage, the haustorium becomes filled with food reserves and its tissue becomes loose, with intercellular spaces. The haustorium penetrates the cortex and comes into contact with the vascular tissue of the host root.

Koch supposed that parasitism must be injurious to the host plant, but had not been able to observe any ill effects caused by it apart from damage to the roots.

Work of R. von Wettstein.

Wettstein (1896) observed that the roots of the whole root system are extremely fine, and that root hairs are noticeably few, occurring singly near root tips and on
haustoria, and in a dense ring at the base of the hypocotyl, where they play a part in the initial anchorage of the seedling. He reported that his observations on the anatomy of the haustoria of *E. rostkoviana* agreed fully with those of Koch on "*E. officinalis*".

Wettstein transferred a seedling of *E. rostkoviana*, germinated on blotting paper, to distilled water. Only one pair of leaves became visible. Seedlings germinating in troughs in the open, in which grass seed had been sown the previous year, produced quite vigorous plants which flowered and had numerous haustoria. With no hosts the plants were dwarf, produced up to ten pairs of leaves, formed no flowers and had no haustoria.

Wettstein carried out an experiment in which a seedling of *E. rostkoviana*, which had formed several haustoria on a host plant, was detached and placed with its taproot in contact with a grass root. A haustorium developed at the point of contact in nine days.

Wettstein's conclusions were that parasitism is necessary for full development and for flower and fruit formation, and that the initiation of haustoria is dependent on the proximity of a suitable host root.

The first of these conclusions soon had to be modified as a result of further experiments (Wettstein, 1897). In experiment A there were 15 pots with root-free garden soil. Four *Euphrasia rostkoviana* seeds were sown in each, 3 cm. apart, in October 1896. 42 of the 60 seeds germinated the
following March; 36 had died by 1 May, having produced up to three pairs of leaves, and four more had died by 1 July, with up to five leaf-pairs; the remaining two flowered in July; both were weak, and one was chlorotic; they were dug up on 15 July and had no haustoria. In B, 20 E. rostkoviana seedlings, germinated in sand in March, were pricked out isolated in root-free soil on 1 April. 12 were dead by 15 May, 14 by 1 June, and 17 by 1 July. Of the rest, one flowered on 15 July, though small and weak, and two lived until early August without flowering. In C, seed was sown closely in root-free soil in 10 pots in February. There was abundant germination early in March. The plants remained small and weak and many died; 31 remained on 31 July, all being starved, and many chlorotic, but 12 were in flower. Four of these had haustoria on other Euphrasias, and two were parasitizing a weed; six had no haustoria, but two of these had flowers, and one a capsule.

Therefore, though parasitism is necessary for vigorous development, individuals can flower and fruit without it, or when behaving autoparasitically.

Wettstein reported that the identification of host plants in the field was difficult because the Euphrasia roots are very thin and because, when the Euphrasias are flowering, the host roots are dying, and nourishment continues saprophytically. Wettstein's records of hosts are given in Table 1 according to date of publication.

As a result of the records published in 1896 in the
TABLE 1: HOST SPECIES RECORDED FOR EUPHRASIA
BY WETTSTEIN (1896, 1897)
(Observations are on cultivated plants unless stated.
Synonyms in current use are in brackets.)

<table>
<thead>
<tr>
<th>Euphrasia Hosts Recorded 1896</th>
<th>Hosts Recorded 1897</th>
</tr>
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<tbody>
<tr>
<td>bicknelli</td>
<td>Avena elatior (Arrhenatherum elatius)</td>
</tr>
<tr>
<td></td>
<td>Lolium perenne</td>
</tr>
<tr>
<td></td>
<td>Poa nemorialis</td>
</tr>
<tr>
<td>minima</td>
<td>Poa nemorialis</td>
</tr>
<tr>
<td></td>
<td>Lolium perenne</td>
</tr>
<tr>
<td></td>
<td>Poa annua</td>
</tr>
<tr>
<td>hirtella</td>
<td>Poa nemorialis</td>
</tr>
<tr>
<td>rostko-</td>
<td>Agrostis vulgaris (A. tenuis)</td>
</tr>
<tr>
<td>viana</td>
<td>Dianthus carthusianorum</td>
</tr>
<tr>
<td></td>
<td>Trifolium pratense</td>
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<tr>
<td></td>
<td>T. repens</td>
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<tr>
<td></td>
<td>Galium mollugo</td>
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<tr>
<td></td>
<td>G. polymorphum</td>
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<tr>
<td></td>
<td>Erigeron canadensis (Conyza canadensis)</td>
</tr>
<tr>
<td></td>
<td>Carex flava</td>
</tr>
<tr>
<td></td>
<td>C. sylvatica</td>
</tr>
<tr>
<td></td>
<td>Agrostis vulgaris (A. tenuis)</td>
</tr>
<tr>
<td></td>
<td>Avena elatior (Arrhenatherum elatius)</td>
</tr>
<tr>
<td></td>
<td>Festuca ovina</td>
</tr>
<tr>
<td></td>
<td>Lolium perenne</td>
</tr>
<tr>
<td></td>
<td>Poa nemorialis</td>
</tr>
<tr>
<td></td>
<td>Triticum repens (Agropyron repens)</td>
</tr>
<tr>
<td>salis-</td>
<td>Carex alba</td>
</tr>
<tr>
<td>burgensis</td>
<td>Carex sp., wild</td>
</tr>
<tr>
<td></td>
<td>(Failed in cult. on Gramineae)</td>
</tr>
<tr>
<td>stricta</td>
<td>Grew in turf with species of Festuca abundant</td>
</tr>
<tr>
<td></td>
<td>Agrostis vulgaris (A. tenuis)</td>
</tr>
<tr>
<td></td>
<td>Avena elatior (Arrhenatherum elatius)</td>
</tr>
<tr>
<td></td>
<td>Festuca ovina</td>
</tr>
</tbody>
</table>
Monograph, Wettstein suggested that monocotyledons were the principal hosts of *Euphrasia* but that his researches were to continue. It is still quite commonly thought that Cyperaceae and Gramineae are the principal, or even the only, hosts of *Euphrasia*; this view presumably owes its origin to Wettstein's Monograph.

Wettstein (1896) reported a case of damage to the host plants by *Euphrasia*. In a field in the Prague botanic garden, a patch on which *Euphrasia* grew in quantity in 1893 appeared relatively bare of vegetation in 1894. Wettstein does not, however, describe the patch in 1893 and 1892, and it is conceivable that it was relatively bare before 1893 and that it was for that reason that *Euphrasia* grew there so well in that year.

**Work of E. Heinricher. (a) Without Host-plants.**

Heinricher's four papers on "The Green Semiparasites" describe a lot of work on *Euphrasia*. It is necessary, however, to begin by describing an experiment with *Odontites odontites* (*Q. verna*) (Heinricher, 1898 a). Seed was sown in a pot on 2 March 1895, and germination started on 12 April. The largest plants were 2.5 to 3 cm. tall by 27 April. On 29 April, it was found that seedlings of a closely-sown group had haustorial initials at the contact points of their root systems. Two other cultures, thickly-sown, gave the following results: in one there were 36 plants on 2 August, with from 3 to 12 pairs of leaves; two
plants had flower buds and branch initials. On 20 September the strongest plant was 20 cm. tall, had produced 20 flowers, and its lowest capsules were ripe. In the other thickly-sown culture the plants, on 3 August, were from 2 cm. tall, with 4 leaf-pairs, to 7-8 cm. tall, with 2-3 flowers. The plants did not grow much more, and produced up to two or three fruits each. The wide individual variation of the first culture is attributed to a long period of germination, enabling some plants to get ahead by parasitizing others; the uniformity of the second is attributed to germination taking place over a short period. These results are similar to those obtained by Koch with uneven and even sowings respectively, using Euphrasia seed.

In addition to Odontites, Heinricher (1898 a) got Euphrasia stricta to flower with no host in a thickly-sown pot of garden soil. Germination began on 17 March 1896, and 70 plants were present on 15 May. Some plants that were surrounded by many others grew more strongly, and the strongest one started to flower on 8 August when 32 mm. tall. Heinricher pickled it. Eight more were in flower on 13 September. The smallest flowering plants had stems 7-10 mm. tall, their leaves were scarcely half the area of those of the largest, and their flowers were $\frac{1}{2}$-1 the size. They resembled dwarf plants of E. minima seen with no other plants among them on stony slopes. Two other pots were sown, one of which had 36 seedlings by 15 May, and the other, a small one, had 30 by the same date. In neither
did any plants come into flower. Heinricher had one pot in which only one seed germinated; it germinated on 4 February 1897 in a greenhouse, and died on 27 March after producing three pairs of leaves.

Two seedlings of *Euphrasia rostkoviana* in one pot lived only four weeks. More experiments were made later with *E. rostkoviana* (Heinricher, 1898 b). In one pot the seedlings were abundant, and in places dense, on 17 March; the first flower bud was seen on 6 May on a plant with eight leaf-pairs. Some plants died back later in May, and no flowers opened; the pot was put outdoors under a glass roof on 29 May; in June and July iron sulphate and nutrient solution were given. More flower buds were formed in July, and up to 20 leaf pairs were produced, but only one plant eventually flowered, at the end of August, although 80-90 plants germinated. In another pot (Heinricher, 1902) the soil used was *Flussand*, a river sand that is used as a potting soil; the sowing was thick, but only ten seedlings came up; three remained on 14 July, and one flowered on 15 August. In addition, two to three *E. rostkoviana* seeds were sown in each of ten small pots of *Flussand*. In each of five pots one seedling came up; of these, two died in May, one in June, one in July and one in August, all without flowering. These last two treatments were also applied to *E. minima*. Seed was sown thickly on 4 March 1898; 27 seedlings were present on 4 June; they were mostly chlorotic and were attacked by aphides, and none flowered.
The pot was kept and was very mossy in 1899, but 25 seedlings came up in a patch where the moss was thin, and other isolated ones elsewhere. Six dwarf isolated plants flowered in May. Others flowered in July, but they were parasitic on *Oxalis stricta*; they were 8-12 cm. tall (very tall for *E. minima*). Heinricher (1898 b) also sowed *E. minima* outdoors on a plot of gravelly, stony soil. Most plants flowered in June; three examples were illustrated; they were 2 cm. apart from one another but isolated from other plants; they were about 2 cm. tall and began flowering at the third node. They would probably have produced two to four flowers each.

A plant similar to these dwarf ones came up on a plot with Carex sp., but so far away from all plants as to be almost certainly not parasitic. (It, too, resembled wild plants seen on 28 August at Hufnerspiel, Brenner, growing on stony ground distant from other plants). No haustoria were found; lateral roots penetrated rotting wood-fragments in the ground and branched much in them; root hairs were plentiful in places (they are visible in a photograph of the plant).

In 1896 (Heinricher, 1898 b) *E. minima* was grown in a pot in the botanical institute window with no host plant. 27 seedlings germinated by 7 April, and flower buds were present by 19 April. The plants were similar to the dwarf ones outdoors with no host. The flowers produced lacked corolla and stamens, but had a calyx and style. They were
barren. The same symptom was observed by Heinricher in indoor cultures of *E. rostkoviana*; he suggests aphides as the cause.

Heinricher concluded that *E. rostkoviana*, with 1 out of 90 plants flowering with no host, was the most advanced parasite, *E. stricta*, with 9 out of 70 flowering with no host, was less advanced, and *E. minima* was the least advanced. The better results with *E. rostkoviana* in 1898 were attributed to the use of better soil. In fact, Heinricher did not grow approximately similar numbers of plants per pot of each species in the same year and with the same soil. Nor was there any duplication of pots, and his conclusions, though possibly correct, have little foundation. The occurrence of wild populations of *E. minima* with no apparent access to hosts, which Heinricher reports, does however suggest that this species is sometimes autotrophic in nature. Heinricher (1898 b) states that, in having plenty of root hairs and in being relatively independent, *E. minima* resembles *Odontites odontites* (*Q. verna*).

Work of E. Heinricher. (b) With Host-plants in Open Ground.

Heinricher sowed seed of *Euphrasia salisburgensis*, *E. rostkoviana* and *E. minima* in autumn on plots in an experimental ground. At a suitable time Euphrasias and hosts were dug up and the presence of haustoria was demonstrated on the host roots. *E. salisburgensis* was sown on 30 October 1896 on plots prepared with tufts of grass
(Heinricher, 1898a). Seedlings were abundant on all plots on 14 April 1897. Flowering began on 1 June, and reached its peak at the end of June. Haustoria were found on:

- Carex alba
- " firma
- " sempervirens
- " tenuis
- Luzula spadicea
- Avena distichophylla
- Sesleria caerulea
- Poa alpina was also used, but it was not investigated. It was found that the hosts tended to shade out the Euphrasias, so that plants from the middle of dense clumps and on the edges of shade-producing tufts were etiolated, while vigorous development occurred when the Euphrasias were not shaded but were within reach of the host roots. Poa alpina grew very vigorously and shaded out the Euphrasias; Luzula spadicea made a better host than the grasses because of its sparse and less shady growth. Similar variation occurred among the Carex species: C. tenuis was tallest and was the poorest host, C. sempervirens was shorter and a better host, and C. firma was shorter still and the best Carex host. C. alba was too mixed with weeds for certainty that the Euphrasia was restricted to one host.

The following results were also obtained with weeds:

- extraordinarily vigorous growth on Capsella bursa-pastoris;
- vigorous growth on Trifolium pratense;
- better growth on young seedlings than on larger plants of Dipsacus fullonum;
- giant plant on Senecio vulgaris, similar to result
with *Capsella*;

one plant vigorous on *Sonchus laevis*, lower parts shaded by host rosette and etiolated, upper parts normal;

very vigorous development on *Veronica peregrina* and *V. buxbaumii* (*V. persica*).

In some cases a *Euphrasia* plant was found to have attachments to more than one host plant simultaneously, in one case, for example, to *Carex alba*, *Poa annua* and *Trifolium pratense*.

Some sowings made on 30 October 1896 were made on plots sown with hosts on 20 June 1896. On these hardly any Euphrasias flowered, apart from a few weak etiolated ones at the edges of the plots, because the growth of the hosts was too dense by the time the Euphrasias began to grow.

Similarly, *E. rostkoviana* was sown in the autumn of 1896 on plots which had been sown with hosts in the summer. The host plants sown were *Carex sempervirens*, *Arrhenatherum elatius*, *Avena flavescens* (*Trisetum flavescens*) and *Poa pratensis*. The grasses grew too thickly, and only one Euphrasia appeared (on the edge of the *A. flavescens* plot). *Carex sempervirens* produced small isolated tussocks, and the Euphrasia grew vigorously on it. Some plants of *E. rostkoviana* were established on weeds, and some seed blew across to the *E. salishburgensis* plots; *E. rostkoviana* was thereby shown to be growing vigorously on *Carex alba* and on *Calamagrostis* sp., very vigorously on *Poa annua* and *Calamagrostis* sp. simultaneously, vigorously on *P. annua*,...
and vigorously or very vigorously on *Veronica peregrina*. *Carex sempervirens* and *Dipsacus fullonum* also acted as hosts to vigorous Euphrasias, in combination with *Veronica peregrina*.

*E. minima* grew outdoors on hosts as follows:

- *Veronica peregrina*, giant plant;
- *Luzula spadicea*, vigorous;
- *L. spadicea* and *Carex* sp., vigorous;
- *Anthoxanthum odoratum*.

**Work of E. Heinricher. (c) With Host-plants in Pots.**

About 100 seeds of *E. stricta* were sown in a large pot of garden soil with seed of *Avena (Trisetum) flavescens* on 27 February 1896 (Heinricher, 1898 a). *Stellaria*, *Capsella* and *Polygonum* came up as weeds, and most Euphrasia plants were closer to weeds than to *Avena*. Some plants evidently became established; the number of flower initials formed ranged from 1 to 16. The weakest plants were weaker than the strongest in the cultures with no host.

In his second paper, Heinricher (1898 b) states that he grew his pot cultures in south-facing windows of the botanical institute, and that results were poor because of lack of light, attacks by aphides, and the difficulty of correct watering. However, the following results may be quoted for *E. rostkoviana*: one vigorous plant on *Dactylis glomerata*; formation of haustoria and the flowering of weak plants on *Moehringia trinervia*; two plants grew 12-13 cm.
tall, and flowered, on *Capsella bursa-pastoris*.

**Work of A. Fraysse.**

Fraysse (1906, p.99) observed haustoria of *E. officinalis* in fields in Savoie in September attached to the roots of various Gramineae, Compositae and Leguminosae, and to the rhizoids of mosses, etc. He found autoparasitism (and parasitism) commonly in a relative of *Euphrasia*, *Odontites rubra* var. *serotina* (*O. verna* var. *serotina*) (1906, p.89).

Regarding penetration by haustoria, Fraysse found that in *Trifolium* and *Taraxacum* they usually penetrated the cortex but reached the host xylem only exceptionally (1906, p.99), the parasite in this case being *E. officinalis*.

Fraysse tested the haustoria and adjacent regions of host and parasite for starch (1906, p.92). The roots were collected in August; some from plants which had not yet started to flower, and others from freely-flowering specimens. The roots of *Ranunculus repens*, when tested with iodine, showed a regular blue-violet ring in transverse section in the inner cortex, endodermis and first sub-epidermal layers, indicating the presence of starch. In the neighbourhood of a haustorium, the quantity of starch diminished, and the limits of its distribution were less definite; at the level of the haustorium, it disappeared entirely from the invaded region, and was found only on the opposite side of the root. In this investigation the parasite was *Odontites*, and similar observations were made
with this parasite and the host-plant *Trifolium repens*. The underground organs of Monocotyledons do not store starch, so that with them iodine did not give these reactions. In Monocotyledons the haustoria always reached the centre of the root, and sometimes divided the entire stele.

Frasse also investigated the distribution of glucose, using Fehling's solution. He found that, when *Trifolium* and *Ranunculus* were the host plants of *Odontites*, reducing sugar was present in the haustoria in the basal absorbent region, in the central meristematic zone, around the vascular strands, and in the apical region, but not in the peripheral parenchyma; there was more reducing sugar at the apex than at the base. From this distribution of reducing sugar, Fraysse concluded that glucose was passing from host to parasite. The roots of the grasses collected were poor in glucose as well as in starch, and on them the haustoria of *Odontites* contained little carbohydrate: consequently, Fraysse concluded that they obtain from grasses only water and whatever solutes it contains.

Haustoria of *Odontites* on *Leontodon autumnalis* behaved as in *Ranunculus* and *Trifolium*.

In the haustoria of *Euphrasia* Fraysse found the same physiological mechanism as in *Odontites*. He states that the haustoria were constituted only for obtaining carbohydrate, but mentions the presence of abundant protein granules in *Euphrasia* haustoria on *Trifolium* and *Taraxacum*. 
Work of H. W. Pugsley.

Pugsley's only contribution to the study of parasitism in *Euphrasia* (1933) describes the growth of three successive generations of *E. brevipila var. notata* in a pot containing a plant of *Hieracium lingulatum*. Pugsley states that these *Euphrasias* grew well but that "there was certainly no connection between the roots of my seedlings and those of the Hawkweed, which was the only other plant at any time in the pot". He therefore concluded that "some *Euphrasias* can maintain their existence without the help of a host-plant".

Work of G. Neidhardt.

This work was done on *E. rostkoviana* (Neidhardt, 1947). The numbers of plants per pot were not given, but numbers of seeds sown were, and germination was up to 40%. Neidhardt tried 1, 2, 5 or 100 seeds per pot with no host, and 1, 100 and over 100 with *Poa annua*, and over 100 with *P. nemoralis* and *Agrostis vulgaris* (*A. tenuis*). With no host, no *Euphrasias* flowered; when there were 100 in a pot some grew more strongly if in a group of several close together; with 1 per pot plants attained a height of 1 to 3 cm., had up to 10 pairs of leaves and were chlorotic; with 2 and 5 per pot they were similar but reached 5 to 6 cm. with 14 to 16 leaf-pairs. With host-plants present and many seedlings in a pot, the *Euphrasias* became more vigorous after four weeks. If the host was regularly cut back,
plants reached 25 cm. and began to flower at the beginning of August. If it was not cut back, the Euphrasias developed slowly, and died in June without flowering. With one seedling per pot and Poa annua the Euphrasias were vigorous, much-branched and up to 15 cm. tall, and they began to flower at the beginning of August.

Neidhardt also got E. rostkoviana to grow in grass in the garden of the Hamburg-Fuhlsbuttel school, in a part of Germany where the species is not native. Germination took place in April, plants were similar to those of pot cultures and they flowered at the beginning of August. Success was not achieved in all cases, and Neidhardt states that the ground should not be too solid, and should be as moist as possible, and that the hosts should be as low-growing as possible and not too dense.

Work of Crosby-Browne.

Crosby-Browne (1950) was able to record the following as hosts to E. salisburgensis in the mountains of Abruzzi, Italy:-

- Dryas octopetala
- Helianthemum grandiflorum
- Saxifraga aizoon
- Draba aizoides
- Salix retusa
- Silene acaulis
- Trinia glauca
- Thymus subcitratus

The Euphrasia plants were 3 cm. high on the average, and the roots were traced to a depth of 10 cm. All were flowering. Typical Euphrasia plants were also found up to
30 cm. distant from any evident plant material, living or dead, on or below the soil surface.

Summary

Germination.

This takes place in soil or in petri-dishes, regardless of the presence or absence of other species. It is recorded for the months February to June. The earliest sowings do not germinate until February. Sowings in late January and subsequently germinate in 1\(\frac{1}{2}\) to 3 weeks (5 to 7 days in petri-dishes, according to Neidhardt). Sowings after the end of February may result in partial or complete postponement of germination until the normal germinating time the next year. Heinricher obtained germination by sowing in the second spring after the seed was formed; Wettstein and Neidhardt did not succeed in this. Sowings indoors by Heinricher and Wettstein, and at controlled temperatures by Neidhart, germinated normally. Up to 70% germination is recorded.

Other Aspects.

Euphrasia roots form haustoria on the living roots of a wide variety of herbaceous and subshrubby plants. Attacked roots usually die subsequently. The haustoria penetrate to the stele, but Fraysse says that only the cortex is penetrated in very starchy roots; in these, starch becomes less concentrated towards the haustorium. Euphrasias can be
cultivated in the open ground or in pots. They fail if the host gives too much shade and causes etiolation. Flowering and fruiting were rarely obtained in the absence of a host-plant; this was especially the case when the Euphrasias were isolated. Where there are a number of Euphrasias in a pot the individuals parasitize one another, and dominating examples may arise which flower and seem capable of fruiting. Some of Heinricher's conclusions about growth in the absence of a host are criticized; force is given to these criticisms by the marked effect of soil variation on Euphrasias growing with no host, to be reported later. In fact there was some controversy between Wettstein and Heinricher on the behaviour of some Euphrasia species growing with no host, and it no doubt arose from generalisations based on insufficient data.
OBSERVATIONS ON THE DEVELOPMENT OF EUPHRASIA

This part of the thesis covers germination, the morphology of the root system, and the development of Euphrasia in cultivation.

I. Germination

Germination Trial, 1954-5.

This trial was intended to compare the germination of seed samples sown at different dates, and of samples kept in the greenhouse from the time of sowing or put in cold frames for varying periods before being brought into the greenhouse, or kept throughout in cold frames.

Seed of two species was used; they were as follows: E. occidentalis (E351), Perranporth, Cornwall, 10 July 1953, and E. nemorosa (E421), Friday Street, Surrey, 26 September 1953.

The initial conditions of storage for the seed of the two species were slightly different, but most of the time both species were kept successively in a cupboard in a potting shed, the door of which was usually open, and in a living room, occupied and heated (by a gas fire) only outside normal working hours; when this room was unoccupied the window was usually open.

The first sowing was made on 9 November 1953, and the rest at approximately monthly intervals until 15 February 1954. (See Tables 2 and 3).
### TABLE 2: GERMINATION OF E. OCCIDENTALIS (E351)

(25 seeds per pot)

<table>
<thead>
<tr>
<th>Date Sown</th>
<th>9/11/53</th>
<th>8/12/53</th>
<th>13/1/54</th>
<th>15/2/54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pot No.</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Time Outside (wks.)</td>
<td>0 4 9 11 12</td>
<td>0 5 9 13 14</td>
<td>0 6 10 12 14</td>
<td>0 7 11 13 14</td>
</tr>
<tr>
<td>No. Germ. Jan. 1954</td>
<td>0 0 1 2 2</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Feb. 1954</td>
<td>15 15 19 10 0</td>
<td>0 6 5 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Mar. 1954</td>
<td>1 0 0 8 2 0 1 5 4</td>
<td>0 0 1 0 0 0</td>
<td>0 0 1 0 0 0</td>
<td>0 0 1 0 0 0</td>
</tr>
<tr>
<td>Apr. 1954</td>
<td>0 0 0 0 1 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Total 1954</td>
<td>16 15 20 20 5 6 6 5 4 0 0 2 0 0 0 0</td>
<td>0 0 2 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Germ. Apr. 1955</td>
<td>- - 0 - 0 - - - - - - 2 2 0 1 5 0 1</td>
<td>2 2 2 1 5 0 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand total</td>
<td>- - 20 - 5 - - - - - - 2 2 2 1 5 0 1</td>
<td>2 2 2 1 5 0 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the night of 24 January 1954 until 28 January the outdoor temperature did not rise above 0°C. Hard weather continued until 11 February, when a thaw started. The conditions intended for the experiment were not fully realised, since during this cold spell the heating of the greenhouse, always weak, failed, and freezing temperatures occurred in it. The pots were transferred to a properly heated greenhouse on 7 February.

In the following June, some pots from the greenhouse were put outside in a plunge bed, together with those that had remained in the frames all the spring. All these pots
TABLE 3: GERMINATION OF E. NEMOROSA (E421)
(25 seeds per pot)

<table>
<thead>
<tr>
<th>Date Sown</th>
<th>9/11/53</th>
<th>8/12/53</th>
<th>13/1/54</th>
<th>15/2/54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pot No.</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Time Outside (wks.)</td>
<td>0 1/2</td>
<td>9 1/2All</td>
<td>0 5 1/2All</td>
<td>0 1/2AllAll</td>
</tr>
<tr>
<td>No. Germ. Jan. 1954</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb. 1954</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 1954</td>
<td>17 16 12 11 2 12 6 4 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr. 1954</td>
<td>0 0 0 1 7 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 1954</td>
<td>17 16 12 2 11 6 4 0</td>
<td>0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Germ. Apr. 1955</td>
<td>5 - - - 1 - -</td>
<td>4 7 7 2 1 11 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand total</td>
<td>22 - - 10 - -</td>
<td>14 7 7 2 1 11 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

in the plunge bed were then kept for observation in 1955, and they were all pots in which no seeding had taken place in 1954, although the plants in some had flowered. The germination that took place in 1955 in these pots is shown in Tables 2 and 3. The entry in Table 2 of "71" in pot 4 sown on 8 December 1953 means that a seedling came up but that it was not certain whether it was a Euphrasia seedling or not.

The following remarks are based on the results shown in Tables 2 and 3:

1. In both species, early sowing resulted in better
germination, possibly because the seeds sown early were in storage for a shorter time.

2. In *E. nemorosa* germination was poorer the longer the pots were outside in 1953-4, regardless of whether germination actually occurred in 1954 or 1955. It is difficult to suggest an explanation for this, as conditions outside ought not to be unsuitable for the survival of seeds. The seedlings were counted twice a week, so that there was virtually no opportunity for seeds to germinate and then die without being detected, unless they were being eaten.

3. Late sowing led to the postponement of germination until 1955. However, in *E. nemorosa*, some germination in 1955 occurred also in the sowings of November and December 1953.

4. Table 2 shows later germination in pots which remained outdoors until the time when germination had begun indoors. Probably germination does not become possible until a certain date, after which it takes place if the temperature is high enough. Pot 4 of the November sowing of *E. occidentalis* contained only two seedlings from 21 January to 18 February. It was brought into the greenhouse between 15 and 18 February. By 22 February there were 5 seedlings, and by 25 February there were 11; one other had died, so that the total was 12. Further seedlings came up in March. Likewise, in *E. nemorosa* germination in pots brought indoors in February began and finished later,
but this is not completely shown by the monthly figures given in Table 3.

5. The highest percentage germination recorded for *E. occidentalis* was 80, and for *E. nemorosa* it was 88.

**Germination of 1951-1952 Sowings.**

1. *E. pseudokerneri* (E42) was collected on 11 October 1951, and an unknown quantity of seed was sown in each of 10 pots on 6 November 1951 and placed in the greenhouse. Eight pots (nos. 3 to 10) were placed out of doors on 26 November. Frosts occurred shortly afterwards, and severe and frequent frosts occurred in January and early February 1952. The first seedling to be detected was found in late January in pot 1 (in the greenhouse). The pots outside were brought into the greenhouse on 4, 8 or 12 February. The germination of seed is shown in Table 4.

**TABLE 4: GERMINATION OF E. PSEUDOKERNERI IN 1952**

<table>
<thead>
<tr>
<th>Pot Number</th>
<th>Not Outside</th>
<th>Outside 6 Nov. to 4-12 Feb. (10 to 11 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Germinating Jan.</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; Feb. 1-14</td>
<td>5 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; Feb. 15-29</td>
<td>0 0 8 6 10 2 2 1 2 1</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; Mar. 1-14</td>
<td>0 3 0 1 2 6 0 0 5</td>
<td></td>
</tr>
<tr>
<td>Total No. Germinating</td>
<td>5 5 11 6 11 4 8 1 2 6</td>
<td></td>
</tr>
<tr>
<td>Days Inside Until First Seedling Seen</td>
<td>17 11 10 17 17 18 15</td>
<td></td>
</tr>
</tbody>
</table>
There was no germination after 8 March, the seeds having taken up to 28 days to germinate after being brought into the greenhouse.

Ninety more seeds were sown on 19 February 1952 in sand in three pots, and placed under lights in a laboratory. Only one seed germinated up to 22 April; it appeared on 20 March.

An additional 81 seeds were sown on 1 April. The pot containing them was placed in a cold frame. No germination could be detected on 22 April or 6 May.

2. *E. brevipila* (E53) was collected on 5 September 1951, and was treated exactly as the first sowing of *E. pseudokernerii*, (p. 30). There was no germination.

3. *E. occidentalis* (E35) was collected on 11 September 1951. Ten seeds were sown on moist filter paper in each of two petri-dishes on 22 November 1951. Both dishes were kept in the laboratory, but one was given an initial period of a week in the deep freeze at -12 to -13°C. One seed of the unfrozen set germinated on 25 February 1952, but none of the frozen set did. The same treatment was applied to seven other species. No seeds of these germinated. Many seeds were attacked by mould, but a number were still unaffected on 2 April.

4. Samples of from 50 to 150 seeds, representing several species, were sown on 1 April 1952. About half were kept in the greenhouse, and the rest outside. No germination could be detected on 22 April or 6 May 1952.
All the seed used in the 1951-1952 sowings had been stored in the laboratory, which was very well heated and became particularly hot overnight. The atmosphere seemed to be very dry.

The results for the first sowing of *E. pseudokernerii* (p. 30) show that germination of seeds collected as late as 11 October can take place with or without exposure to low temperature. The three sowings of *E. pseudokernerii* (pp. 30 & 31) together suggest declining viability in storage. On the other hand, results from later years indicate that seed sown very late may not germinate until the following year. Sometimes, however, there is a partial germination in the first year, as in the germination trial (pp. 27 & 28). The total failure of all the April 1952 sowings (p. 31) strongly suggests that the seed was by that time inviable. The seed of *E. brevipila* was kept in the heated laboratory two or three weeks longer than the seed of *E. pseudokernerii*, and this might account for the apparent complete loss of viability of *E. brevipila*.

**Germination of 1952-1953 Sowings.**

All seed collected in 1952 was extracted from the plants as soon as possible and kept in a refrigerator at about 8°C. in plastic bags sealed with cellulose tape.

Some samples were sown partly in pots kept in the greenhouse and partly in pots in a cold frame or outdoor plunge bed. The results for all these are given in Table 5.
My records indicate that the counting of seedlings outside stopped before germination ended. The number germinated outside, therefore, is probably underestimated when the date of the last record is an early one.

Taking the samples in the table in order, it will be noticed that E227 gave poor germination inside and out; E192 germinated well inside and out; E166 and E210 germinated better outside than in; E196 germinated well inside and out, as the figure given for its outside germination is

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Species</th>
<th>Number Germ. Inside</th>
<th>Last Record</th>
<th>No. Germ. Last Outside Record</th>
</tr>
</thead>
<tbody>
<tr>
<td>E227</td>
<td>E. foulaensis</td>
<td>11</td>
<td>2 Apr.</td>
<td>10</td>
</tr>
<tr>
<td>E166</td>
<td>E. nemorosa</td>
<td>4 out of 100</td>
<td>24 Mar.</td>
<td>120</td>
</tr>
<tr>
<td>E210</td>
<td>E. nemorosa</td>
<td>3 out of 150</td>
<td>24 Mar.</td>
<td>300-400</td>
</tr>
<tr>
<td>E209</td>
<td>E. confusa</td>
<td>5</td>
<td>2 Apr.</td>
<td>10</td>
</tr>
<tr>
<td>E226</td>
<td>E. pseudokerneri</td>
<td>6</td>
<td>2 Apr.</td>
<td>300-400</td>
</tr>
<tr>
<td>E150</td>
<td>E. anglica</td>
<td>0 out of 75</td>
<td>24 Mar.</td>
<td>4</td>
</tr>
<tr>
<td>E187A</td>
<td>E. anglica x micrantha</td>
<td>4</td>
<td>2 Apr.</td>
<td>16</td>
</tr>
<tr>
<td>E123</td>
<td>E. rivularis</td>
<td>0</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>E230</td>
<td>E. salisburgensis var. hibernica</td>
<td>2</td>
<td>2 Apr.</td>
<td>3</td>
</tr>
<tr>
<td>E232</td>
<td>E. sp.</td>
<td>0</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>
almost certainly one of the underestimates; E209 gave very poor germination inside and out, for in both cases the number of seeds sown was quite substantial; E226 gave very little germination inside, in spite of a substantial sowing of seed, whereas the germination outside was very good; E150 gave much better germination outside than in, for the figure given for its outside germination is almost certainly another underestimate; the last four samples gave somewhat better germination outside than in.

Germination in general in 1952-53 seemed to occur more readily outside than in; for, out of 23 miscellaneous samples of seed sown inside, germination occurred in only 12, whereas 14 out of 52 samples gave germination outside.

Some samples of seed produced by enforced self-pollination in 1952 were sown in pots in the heated greenhouse on 20 December 1952. The pots in which no germination had taken place were plunged outdoors under a tree on 16 May 1953. In September 1953 the outside pots were taken from Leicester to Cambridge, and were placed temporarily in a greenhouse. They were plunged in ash out of doors in October. In February 1954 the layer of moss covering the pots was broken up with a penknife. On 13 April the pots were transferred to a cool greenhouse. The germination of the samples is shown in Table 6, p.35. The seeds produced by artificial hybridization (which, as it turned out, included a few resulting from accidental selfing) in 1952 were treated in the same way. The results were similar: 4
TABLE 6: GERMINATION OF EUPHRASIA SEED IN GREENHOUSE IN 1953, AND IN 1954 AFTER WINTERING IN OPEN

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Species</th>
<th>No. of Seeds Sown</th>
<th>Mar &amp; April 1953</th>
<th>Mar &amp; April 1954</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E73</td>
<td>E. occidentalis</td>
<td>47</td>
<td>3</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>E160</td>
<td>E. nemorosa</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E70</td>
<td>E. pseudokerneri</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>E42</td>
<td>E. pseudokerneri</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>E71</td>
<td>E. anglica</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Seedlings appeared in 1953 and 68 (or slightly more) in 1954. One pot, which produced 2 seedlings in 1953, produced a further 3 in 1954.

Discussion of 1952-1953 Sowings.

The results show that it is unusual, though sometimes possible, for germination to be very good in seeds kept in the greenhouse throughout the winter. More frequently, germination is poor indoors when it is very good outside, or it fails inside when it is not very good outside.

Germination in two successive springs, or only in the second spring after sowing, is again demonstrated (cf. pp. 27 & 28) in seed samples kept indoors during the first winter.

Germination of 1953-1954 Sowings.

The 1953 seed was stored in plastic bags in a cupboard.
in an entrance hall, the door of which was nearly always open by day and shut at night.

Germination tended to be poor. On 8 December 1953, 19 samples of wild seed were sown, and placed in plunge beds in the open. Germination occurred in 15 of the samples, but only two produced more than 40 seedlings, although 11 or 12 samples probably contained substantial quantities of seed. A few seeds germinated in February, but the germination of the majority was evenly divided between March and April.

14 samples were sown in pots on 17 February 1954, and placed outdoors in a plunge bed. Germination took place in 8 samples, and was poor in most of these; the number of seedlings per pot was 2 to 46 or more. Six of the pots, in one of which germination had already taken place, were kept outdoors over the following winter, and germination took place in all in 1955, 2 to 19 seedlings appearing per pot.

Another group of 18 samples was sown in pots on 15 February 1954, and placed outdoors in a plunge bed. The seeds were produced by artificial cross- or self-pollination in 1953. Germination for the group as a whole is given in Table 7, p.37. In 1954, germination took place in 8 pots. All the pots were kept outside during the following winter, and germination took place in 9 of them in 1955, 4 pots producing seedlings in both years.

A sample of seed of *E. nemorosa* was sown on 17 February 1954. Probably about 90 seeds germinated in 1954,
TABLE 7: GERMINATION OF EUPHRASIA SEED SOWN ON 15 FEBRUARY 1954

<table>
<thead>
<tr>
<th>Date</th>
<th>Number Germinating 1954</th>
<th>Number Germinating 1955</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-31 March</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>1-15 April</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>16-30 April</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>1-31 May</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19</strong></td>
<td><strong>82</strong></td>
</tr>
</tbody>
</table>

about 200 in 1955, and one in 1956. It is believed that no seedlings flowered in the seed-pot in 1954 and 1955, so this single 1956 plant was presumably from the original sample.

**Discussion of 1953-1954 Sowings.**

The behaviour of the seed was similar to that of previous years. The generally poor germination may have been caused by a prolongation of cold weather and then a quick change to dry sunny weather. The cold weather would have held up germination, and the dry weather may have kept the surface of the pots too dry for germination much of the time. An attempt to improve the watering of the pots and to embed them better was made in mid-April 1954.

**Germination of 1954-1957 Sowings.**

The germination of sowings in this period was again similar to that of previous years; it failed or was
incomplete if the sowing was late. The following samples are worthy of particular mention. A sample of *E. tatrae* (E514), although sown as early in 1955 as 20 January, gave no germination in that year, but three seedlings appeared in 1956. On the other hand, a sample of *E. rostkoviana* (E530), not sown until 14 April 1955, produced two seedlings in the latter half of May 1955. In 1956 the pot was overlooked until 1 May, when eight seedlings were present. The 1955 germination of this sample is among the latest that I have encountered, and 14 April is the latest sowing date which has given germination in the season of sowing in my experiments. In 1956, seed of *E. stricta* (E622) was sown on 15 March. The beginning of germination was overlooked, and on 24 September the pot contained a plant of *Trifolium repens* and three plants of *Euphrasia* with ripe seed. In addition, two newly germinated seedlings of *Euphrasia* were present, one with only the first pair of leaves visible, and one with three pairs visible; a third seedling, with two pairs of leaves, was detected on 26 September. It seems likely that these seedlings germinated in September. They had all died by 20 November, although the pot had been brought into the greenhouse, and the mature plants pressed, on 24 September.

In the winter of 1954-5, several samples of seed, produced in cultivated plants two and a half years earlier, were sown. There was no germination.

In this period 1954-7, all the seed was kept out of
doors from the time of sowing at least until the time of germination.

Summary of Germination Results.

Under dry conditions of storage the seed tends to lose its viability. Germination is recorded for the months January to May, and probably, but exceptionally, for September. Early sowings do not germinate before January. Seed that had probably become able to germinate took 10 to 28 days to germinate on being brought from cold outdoor conditions into a warm greenhouse (pp.30-31). Partial or complete postponement of germination until the spring of the following year occurred with certain sowings outdoors from 20 January onwards. Late germination resulted from late sowings in which germination took place in the same year. Greenhouse conditions after autumn sowing produced poorer germination than outdoor conditions in many samples, but not in all. Good germination occasionally occurred in the greenhouse, but it failed or was poor more often than outdoors. When this was the case, germination usually occurred if the pots were kept outdoors the following winter. No germination was obtained in seed sown in the third winter after its formation. Up to 88% germination is recorded.

These results agree closely with those of previous workers except that lack of exposure to outdoor winter temperatures was unfavourable to germination in my experience. Loss of viability of Euphrasia seed in storage was encountered
in the first and third winters after seed-setting. Earlier workers, other than Heinricher, also encountered loss of viability of Euphrasia seed when sowing seed over one year old.

II. Observations on the Roots of Euphrasia

1. E. pseudokerneri (E42) in 1952.

The plants were grown with various hosts in pots in the greenhouse.

A plant growing with Pelargonium x hortorum was dug up in fruit on 9 May. From its growth, it hardly appeared to be established on the host, but Pelargonium roots were seen where the seedling was dug up. Slightly less than one inch of the seedling's tap root was extracted; it had branches, and these bore haustoria. One piece of host root came away attached to a haustorium; it appeared to be dead, which would account for its coming away with the parasite.

Euphrasia roots are very fine and easily broken, and the haustoria usually come away readily from the host.

A plant with Plantago lanceolata and Prunella vulgaris had died back progressively until, on 14 May when it was dug up, only the cotyledons remained green. It had long portions of unbranched root, but distally two branches of a fork were more branched and closely applied to a dead root; a few haustoria were developed here.

A plant with Koeleria gracilis was dug up on 5 June.
This was also a small plant. No haustoria were seen, but the lateral roots had broken off. Koeleria roots were abundant around the Euphrasia.


Haustoria were observed in some pots at the time the plants were collected for pressing. The soil ball was knocked out of the pot, and the mass of roots at its periphery examined for haustoria. Attachments to Medicago lupulina were seen on two samples of E. nemorosa (E344 and E417), on E. anglica (E71), on several hybrids, and in abundance on E. salisburgensis (E204) and E. pseudokernerii (E70); in both samples of E. nemorosa, haustoria were also found attached to Euphrasia roots. With E. vigursii (E348) and Medicago lupulina no haustoria could be found, although the only Euphrasia plant was fairly well grown. However, no Euphrasia roots could be found at the periphery of the pot; a few were found in the interior, but haustoria would in any case be difficult to find there.

Haustoria on host roots were found also on E. micrantha (E185A). The intended host was Medicago lupulina, but young plants of Ranunculus sp. and Lamium purpureum were also in the pot at the time of examination.


Haustoria were found connecting E. nemorosa to Hieracium pilosella, and E. nemorosa to itself.

Haustoria attached to Medicago lupulina were found on E. scotica (E603).

5. Garden Cultivation in 1953.

Very vigorous plants of E. nemorosa were grown in the garden in embedded "whalehide" pots (which rot fairly quickly) with Plantago lanceolata as host. The roots of two host plants, together with their parasites, were pulled up and preserved in alcohol. The roots of each host, with its parasite, formed a dense mass in which there were numerous haustoria, but in which it was difficult to make out connections. However, each host was seen to be connected to its parasite by haustoria. In both cases haustoria were seen on large Plantago roots (Fig. 1), and

![Diagram of haustoria and roots](image)

FIG. 1: Roots of E. nemorosa with haustoria attached to a much thicker root of Plantago lanceolata.
in one case on relatively thin ones.


Sets of seedlings, germinated in pots and representing five samples (four species and one doubtful population), were photographed on 1 and 2 April 1953. The photographs (Figs. 2-6) show the development of the taproot and the lateral roots magnified about 1.5 times. The youngest

FIG. 2: Seedlings of E. micrantha (E185A).

FIG. 3: Seedlings of Euphrasia sp. (E231, related to E. micrantha).
seedlings and a number of leaves. Recently it was apparent that the variation in seedlings resembled E. occidentalis (E226) were like those of E. occidentalis (E192). The seedlings of E. rostkoviana (E233) were like E. occidentalis (Fig. 4). At the base of the branchlets a tuft of leaves develops; these tufts, which are probably necessary to give the seedlings of E. rostkoviana (E233). If these tufts do not appear above the ground, if they never give anchorage, it is not known from such. It was noted that E. anglica (E180) sand is firmly attached to the root system and often appears above the soil.
seedlings are just free of the testa, or have the cotyledons still within it; the oldest show the first pair of leaves. The degree of root-branching in plants of apparently similar age appears to vary from population to population. Seedlings of three other species were photographed, but the ones shown here exhibit all the variation that was present. Seedlings of *E. pseudokerner* (E226) were like those of *E. rostkovi*ana (Fig. 5); those of *E. nemoros* (E215) were like *E. anglica* (Fig. 6); those of *E. confus* (E208) were like *E. occidentalis* (Fig. 4). At the base of the hypocotyl a tuft of root-hairs develops; these tufts can be seen in the photographs. They give the impression of being effective in anchorage, and are probably necessary to ensure that the cotyledons come above ground. If they were absent the seed and cotyledons would give anchorage, and the hypocotyl would probably never appear above ground. Root-hairs are sparse elsewhere on the root system of the seedling; they are usually absent from much of it, and occur in groups here and there (Fig. 7, p. 46). They also seem to develop around haustoria. It was noted that on five seedlings of *E. occidentalis* (E192) sand grains and organic debris were found firmly attached wherever root-hairs were present. The root system often appears to branch more in pieces of peat in the potting compost than elsewhere.

Lateral branches are swollen at their base, and the initial of a lateral branch looks similar to a detached
FIG. 7: Seedling of *E. occidentalis* (E192), grown in greenhouse, drawn on 3 February 1953, four weeks after the cotyledons had appeared above ground.
haustorium (Fig. 7). No haustoria were evident in the seedlings shown in the photographs (Figs. 2-6, pp. 43 & 44), but more mature seedlings collected in the wild showed numerous haustoria. A drawing of one seedling is supplied (Fig. 8, p.48). It was not possible to trace the parasitized roots back to their origins. They were, therefore, cut off near the haustoria. Numerous detached haustoria were also present. The root system of the Euphrasia drawn is incomplete.

A small pot of E. micrantha (E185A) seedlings was investigated on 20 June 1953. The Euphrasias were in the pot in which they had germinated, and no other plants were present. Haustorial connections between roots were found, as shown in Figs. 9 & 10, p.49.

Another drawing of an attachment of a plant to itself is given in Fig. 11, p.50. This was a single plant of E. nemorosa (E507) growing in a pot with no host plant in 1955. Haustorial connections between Euphrasia roots were also found in the seed pot of E. confusa (E208) on 20 June 1953.

Euphrasia seedlings which are not established on host plants show very slow growth above ground, and the leaves they produce are small. However, the root system grows quite actively, and produces an appreciable length of very fine roots. For cultivation it is usually necessary to transplant the seedlings from the pots in which they germinate into pots provided with host plants. The earlier
FIG. 8: Wild seedling of *E. pseudokerneri* (E70), drawn on 1 May 1952. Haustoria shown black, host-roots shaded. There are five haustorial connections.
FIG. 9: **Left** - Two plants of *E. micrantha* (El85A) from a pot of *Euphrasia* seedlings, 20 June 1953, joined together by a haustorium. **Right** - The connecting haustorium, greatly enlarged.

FIG. 10: Roots of *E. micrantha* (El85A), 20 June 1953, connected by haustoria. The drawing on the left shows two branches of the same root connected by a haustorium.
the seedlings are planted out the less they are damaged in
the process, and it is therefore necessary to plant them
out when they are extremely small. In practice it seems
better not to plant them out as soon as they are visible
above ground, but to do so two to four weeks after they
appear. For example, suitable seedlings for planting out
would be the second and third from the left in Fig. 4,
p.44, but the left-hand plant would be rather old for
planting out; the two right-hand ones in Fig. 2, p.43 have
too little root to make it desirable to transplant them.

Summary.

Haustoria were found on quite dwarf Euphrasia plants
growing in pots with other plants, but the only attachments
found were to dead roots. Haustoria were also found on
Euphrasia plants that, by their vigour, appeared to be
established on hosts, and the attachments were in most cases
to live host roots, but occasionally to dead ones. In such
cultures, haustoria connecting two Euphrasia roots were also
found.
Single *Euphrasia* plants can form haustoria connecting two of their own roots. When a number of *Euphrasia* plants were grown with no host plant, haustoria were found, and in one instance it was shown that two separate plants were connected by a haustorium.

Haustoria were quite numerous on wild *Euphrasia* plants observed in early May.

Haustorial connections and seedling morphology are illustrated by drawings and photographs. The young seedling has few root hairs apart from a dense anchoring tuft at the base of the hypocotyl. Growth of plants with no attachment to a host is very slow above ground, but root growth may be quite extensive. For cultivation, therefore, seedlings have to be moved when quite small, or excessive damage to the root system may result.

These results agree well with those of previous workers, but slightly extend them in that autoparasitic unions have been seen in single isolated individuals and in pots where heteroparasitic haustoria were present.

### III. Observations on Euphrasias Growing in Cultivation

**Euphrasias Grown From Seed in 1952.**

A cultivation experiment was made using *E. pseudo-kerneri* (E42 & E60). E42 was a gathering of herbarium specimens made on 11 October 1951 from which seeds were extracted; E60 was a turf, containing dead seeding plants
of *Euphrasia*, collected from the same place on 8 November 1951; it was planted in a pan with extra soil. Batches of the E42 seeds were sown in five-inch-diameter pots, which were provided with hosts as follows:

```
Koeleria gracilis  plants (pot K)
Festuca ovina      " (" F)
Achillea millefolium seeds (" A)
Plantago lanceolata " (" P)
Thymus drucei      plants (" T)
Pelargonium x hortorum plant (" Pe)
Prunella vulgaris  seeds (" Pr)
Festuca ovina & Koeleria gracilis plants (" F & K)
Festuca ovina & Thymus drucei  " (" F & T)
Plantago lanceolata & Prunella vulgaris seeds (" P & P)
No host plant      (" NH)
```

The soil in these pots was loam with a layer of sand on top, except in Pe, NH, and P & P, where the loam and sand were mixed.

The plants accompanying E60 were *Helianthemum chamaecistus*, *Linum catharticum*, *Poterium sanguisorba*, *?Centaurea scabiosa*, *Hieracium pilosella*, *Leontodon sp.*, *?Campamura glomerata*, *Thymus drucei*, *Plantago media*, *Carex carvophyllea*, *C. flacca*, *?Poa trivialis*, *Brachypodium pinnatum*, *Bromus erectus*, *Festuca ovina* and *Koeleria gracilis*.

Seedlings of E42 and E60 germinated in a heated greenhouse between about 28 January and 7 March 1952. The host seed started germinating during the period of germination of the *Euphrasia*, and continued until the beginning of April; *Plantago lanceolata* was exceptional in that some seedlings came up soon after sowing on 6 November 1951.

Failures in germination made it necessary to transfer some young seedlings of hosts and of *Euphrasia* from one pot
to another. Pot P & P failed and a new one was prepared - P & P (2).

Regular recording of the development of plants began on 21 March 1952. The dates of appearance of the following characters of the plants were recorded: leaves, branch initials, flower buds, and teeth on one side of one leaf at each node (the teeth being observed on the leaf-side with the greatest number of teeth at each node). A note was made later to show which branch initials produced perceptible internodes and which flower buds opened.

Since photographs of all the growing plants are not available, and since it is impracticable to illustrate every plant after pressing, an attempt has been made to illustrate the variation in development by using these records.

Diagrams of Plants at End of Recording.

Each plant is represented by a diagram, Figs. 12-15, pp. 54-57. The vertical line represents the number of pairs of leaves visible, each pair being represented by a length of 5 mm. The number of teeth is indicated by the length of the horizontal lines, 2 mm. being equivalent to one tooth. (The range of tooth numbers is 0 to 5). Lines at 45° to the vertical represent branches on which internodes became apparent, and the oblique ellipses represent flowers which opened. The diagrams represent the states of the plants on the last occasion of observation. This was in many cases
FIG. 12: Diagrams of plants of *E. pseudokerneri* (E42). (See pp. 53, 58 and, for list of hosts, 52).
FIG. 13: Diagrams of plants of *E. pseudokerneri* (E42). (See pp. 53, 58 and, for list of hosts, 52).
FIG. 14: Diagrams of plants of *P. pseudokerneri* (E42). (See pp. 53, 58 and, for list of hosts, 52).
FIG. 15: Diagrams of plants of *E. pseudokernerii* (E42). (See pp. 53, 58, for list of hosts, 52).
13 July 1952, but where the last date on which progress was observed was earlier than this it is given below the diagram.

It should be borne in mind that recording stopped long before the plants normally ended their development, and that many of those shown without flowers produced them later. Comparison with the pressed specimens shows that every difference in the degree of development shown by the diagrams corresponds with a similar, but much greater, difference in the plant. This is because leaf size and internode length are not shown in the diagrams, nor is the degree of development of the branches. Leaf size is not strictly proportional to the number of teeth.

If the diagrams of the plants with hosts are compared with those of plants with none, it will be seen that with some hosts the Euphrasias are as small as or smaller than those with no host, while with other hosts some Euphrasias are similarly small and others are much more vigorous, having leaves with more teeth and having produced branches and, in some cases, flowers; they also produced more leaves in a given time. It seems probable that the presence of the host plant is responsible for the vigour of these Euphrasias. It can be seen that quite good development occurred with Festuca ovina, but not with Koeleria gracilis, while the combination of the Festuca with Koeleria gave good development. Presumably K. gracilis is a poor host and F. ovina is a good one and is responsible for the good
growth in F & K. When one of the dwarf plants in pot K was dug up on 5 June, the grass roots were abundant around the roots of the Euphrasia, as described on p. 41. The Euphrasia apparently had the opportunity to benefit parasitically, but its small size shows that it was unable to make use of it. Similarly, good development occurred with Plantago lanceolata but not with Prunella vulgaris, while good development occurred with the combination of Plantago and Prunella. Some attached haustoria were found on the dwarf plant in pot P & P (2) on 14 May. That some plants such as this, with apparently good hosts, do not develop properly could be due to a failure to establish sufficient haustorial connections with the host roots, to the Euphrasia plant's being parasitized by another, or to individual variation in capacity to parasitize particular species. One instance in which the second possibility cannot apply is supplied by the single plant with Festuca and Thymus, which grew feebly although it was the only Euphrasia in the pot. It is evident that negative results in host trials must be regarded with caution. One of the dwarf plants in pot T which was dug up dead on 10 May had Thymus roots around it and bore a few detached haustoria; this is an instance of parasitic attack failing to benefit the parasite. In the whole experiment, the largest plants by far were plant 3 of pot Pe and plant 5 of pot F & K. Plant 2 of pot P & P (2) promised to equal or excel these but became diseased and was pressed on 13 July. An interesting feature of the
cultivated plants, which is shown in the diagrams, is that many of them flowered at an extremely low node. *E. pseudokernerii* normally begins flowering at about the 14th or 15th node, whereas these plants began at nodes from 3 to 15. The earliest node at which I have known a *Euphrasia* to begin flowering is the second. A very early node of flowering characterizes alpine and arctic species such as *E. cambrica*, *E. rivularis* and *E. frigida*. This phenomenon is discussed further in the following section.

Photographs of some of the plants mentioned above are given in Figs. 16-20, pp. 61-63.

**Graphs of Development.**

For each of a number of selected plants two curves are shown (Figs. 21-24, pp. 64-67). The lower curve shows the number of pairs of leaves present with teeth visible on the dates indicated. Every leaf has at least one pair of teeth, but these are not visible at first. Unless the plant is very starved, the number of teeth increases from one pair on each leaf at the first node to a maximum (in this population) of five pairs on some later leaf. The upper curve shows the sum of the number of teeth visible at each node, the number of teeth at a node being estimated as described on p. 53. Though tooth number almost always rises above one, it rises more in the more vigorous plants, and is here used as an indication of vigour. As already stated, tooth number is not strictly proportional to leaf size,
FIG. 16: *E. pseudokerneri* (E42) pot Pl, probably early June 1952. The flexuous-stemmed plant with fruits, in the centre, had been decapitated.

FIG. 17: *E. pseudokerneri* (E42) pot F & K (left), showing plant 5, and pot Pe (right), showing plant 3. About 20 July 1952.
FIG. 18: E. pseudokernerii (E42) pot P & P (2); plant 1 in centre, plant 3 to left, probably second half of July 1952.

FIG. 19: E. pseudokernerii (E42) pot Pe, showing plant 3. 5 September 1952.
FIG. 20: *E. pseudokerneri* (E42) pot F & K, plant 5 the largest. 5 September 1952.
FIG. 21: Graphs of development of plants of *E. pseudokernerii* (E42). (See pp. 60, 68 and, for list of hosts, 52).
Fig. 22: Graphs of development of plants of *E. pseudokernerii* (E42). (See pp. 60, 68 and, for list of hosts, 52).
FIG. 23: Graphs of development of plants of *E. pseudokernerii* (E42). (See pp. 60, 68 and, for list of hosts, 52).
FIG. 24: Graphs of development of plants of *E. pseudokerneri* (E42). (See pp. 60, 68 and, for list of hosts, 52).
which would give a better indication of vigour. The plants that evidently became established on their hosts are (among those plotted) plant 3 of pot Pe, plant 2 of pot F, plant 3 of pot F, plant 2 of pot F & K and plant 5 of pot F & K.

The time required for these plants to show visible signs of establishment can be estimated from the curves. The dates after which their curves differ noticeably from those of the other plants are, respectively, 9 May, 30 May, 16 May, 23 May and 30 May. The times between these dates and the corresponding dates of germination range from 72-95 days, which is very long compared with the shortest times for establishment recorded in later years. Most of the other curves (where a host is present) are similar to that of the largest host-less plant, plant 3 of pot NH. However, plant 4 of pot F and plants 3 and 4 of pot F & K developed later and were finally considerably larger than plant 3 of pot NH, probably becoming belatedly established on their hosts.

Plants 1 and 5 of pot F and plant 1 of pot F & K were finally about the same size as plant 3 of pot NH. Possibly these were also getting some benefit from the host, since if they were not they would merely be in competition with it, and would then be expected to be even smaller than those plants with no host at all. The remaining curves show plants that died or were pressed when about to die, before the end of recording. Plant 6 of pot F measured 3mm. from cotyledons to apex when dead, and it appears to have died of starvation. The others, two in pot NH and three in pot
Pe, began flowering at the third or fifth nodes, produced two or three capsules, and began to die as the seed ripened. The first flower buds were seen on 28 March, and the first flowers opened shortly before 21 April, during my absence from Leicester. The plants completed their life cycle and died in a natural way and not from disease. This precocious flowering was due to the very high temperatures prevailing in March in the greenhouse, which was usually heated and unventilated; at this time there was a period of fine sunny weather which drove up the temperature of the greenhouse to the eighties (°F.). This suggests that temperature is one of the main factors in inducing flowering in Euphrasia, and that given sufficiently high temperatures the autumnal E. pseudokerneri can be made to behave like the early-flowering alpine species of Euphrasia. At the time recording was started, on 21 March, these five plants that flowered and died early had three or four leaves with visible teeth, whereas all the others graphed (none of which flowered precociously) had only 0 to 2. It seems, therefore, that, being at a sufficiently early stage of development, these latter plants were unable to respond to high temperatures by premature flowering and death. Even including the one without a host plant, they were ultimately able to develop more fully than those that in the first place appeared more vigorous.

The precociously flowering plant 4 of pot Pe was found to have its roots surrounded by Pelargonium roots, and they
bore detached haustoria; one piece of dead host root came away attached to a haustorium. Presumably the plant was not sufficiently well established parasitically to stand the drain on its reserves caused by flowering when it was so small. In pot P1, however, some plants flowered rather less precociously, early in May, and at the 5th to 7th node, and these plants survived until July or August.

Development of *E. pseudokerneri* (E60) in Turf.

This turf was grown in a pan (see pp.51-52). On about 8 March 1952 there were approximately 23 seedling Euphrasias present. Plants of *Euphrasia* came up in the turf and in the soil round the edge of the pan outside the turf. In March and April those in the turf developed better than those outside it. By 21 April one plant was in flower, and others flowered by 30 April. On 2 May three were flowering at their 3rd or 4th nodes. The early-flowering plants included some in the turf and some outside it; the former were green, while the more starved peripheral plants had a strong development of anthocyanin (this was also observed in the more depauperate plants of *E. pseudokerneri* mentioned in the preceding section). One of the plants in the turf had a flower 8mm. long, though not fully expanded, on 2 May, while one of the plants outside it had a fully expanded flower only 5mm. long, on the same date.

Ultimately, several quite vigorous plants developed (Fig. 25, p.71), and they tended to be more branched, and
FIG. 25: *E. pseudokerneri* (E60) in a piece of its natural turf, collected on 8 November 1951, and photographed on 5 September 1952.

thus more normal for the species, than the plants of *E. pseudokerneri* discussed in the preceding section. The effects of temperature fluctuations were evident in the plants growing with the turf. As mentioned (p.69) there was a hot period in the greenhouse in March, and there were probably later ones during the spring. After this the greenhouse was ventilated more in proportion to the amount of sunshine, and more natural temperatures prevailed. Two of the plants growing with the turf flowered at two or three nodes from the 6th to the 8th, then produced branches at higher nodes (normal in one plant and poorly developed in the other) and then flowered again. Two other plants,
after producing some low branches, formed flower buds which, however, failed to develop; later the plants produced more branches, and then flowered.

**Cultivation of Wild Euphrasia seedlings in 1952.**

In 1952 numerous naturally germinated seedlings were grown, usually in their own turf, but in some cases separated and provided with rooted pieces of a single host species planted at the same time as the Euphrasias. Among the seedlings were some of *E. pseudokerneri* (E70) collected from the same locality as E42 and E60 (also *E. pseudokerneri*) on 29 April 1952. Recording was carried out as for E42 (p.53) from 10 June to 1 August for some of the plants; diagrams have been prepared (Fig. 26, p.73) showing vigorous growth on *Sieglingia decumbens* (pot S) (there were two plants in the pot, but only one was recorded), *Carex caryophyllea* (pot C), and *Hieracium pilosella* (pot H), while rather poor development took place on *Bromus erectus* (pot B). Figs. 27 & 28, pp.74-75, show photographs of the plants. In addition, one vigorous and one rather poor plant were produced with a mixture of *Dactylis glomerata* and *Brachypodium pinnatum*. The times between potting up and apparent establishment on the host were 40-50 days for pots S and C, and 50-62 days for pot H.

*Euphrasia pseudokerneri* (E66, from a different locality) was also tried on *Poa trivialis* and *Deschampsia flexuosa*. The plants were collected on 15 April and
FIG. 26: Diagrams of plants of *E. pseudokerneri* (E70 & E66). (See pp.72, 75 and 53).
FIG. 27: *E. pseudokernerii* (E70), grown from wild-collected seedlings potted up with host-plants. 5 September 1952. **Left** - One *Euphrasia* with *Hieracium pilosella*. Scale shows mm. and cm. **Right** - Two *Euphrasias* with *Sieglingia decumbens*. 
planted in pots five inches in diameter. Two moderately vigorous but small-leaved plants grew on the Poa, while two others were very weak (pot P, Fig. 26, p.73). Five plants grew with the Deschampsia, three being comparable with the stronger ones on Poa, and two being weaker. E. pseudokerneri (E66) was also tried without a host, and the results were similar to those of E42 (pot NH, Fig. 12, p.54). For photographs of the E66 plants, see Figs. 29-31, pp.76-77.

Finally, the following Euphrasias and hosts were tried and the Euphrasias failed to show signs of establishment after they had stayed alive for some time:
FIG. 29: *E. pseudokernerii* (E66), wild-collected seedling grown with no host. 5 September 1952.

FIG. 30: *E. pseudokernerii* (E66), wild-collected seedlings growing with a grass, probably *Deschampsia flexuosa*; five Euphrasias present. 5 September 1952.
FIG. 31: *E. pseudokernerii* (E66), pot P. Wild-collected seedlings growing with *Poa trivialis*; two Euphrasias present. 5 September 1952.

*E. anglica* (E67), collected on 15 April 1952; there were 9 seedlings in a five-inch diameter pot with two plants of *Plantago lanceolata*; three Euphrasias lived until July or August;

*E. occidentalis* (E73), collected on 3 May 1952; there were one or two seedlings in each of four 3½-inch diameter pots; each pot contained a different host, as follows: *Plantago lanceolata, P. coronopus, Festuca ovina* (*Aira praecox* and *F. rubra* also included accidentally), and *Luzula campestris* agg.; the Euphrasias were still alive on 17 June.

Among plants of *Euphrasia* grown in their own turf, it
appeared that the presence of leguminous plants had a particularly favourable influence. Thus, in a pot of *E. occidentalis* (E73), there were four plants, two in each of two pieces of turf. Plants 1 and 2 were with *Plantago coronopus*, a good deal of *Trifolium repens*, *Thymus*, *Cerastium vulgatum*, probably *Trisetum flavescens* (the main grass), and *Festuca ovina*. They had dark green and shining leaves, whereas plants 3 and 4, mainly with *Holcus lanatus* but also *Festuca*, *Dactylis glomerata*, *Sagina procumbens*, *Cerastium vulgatum* and *Leontodon levsseri*, had leaves of a lighter green. Plant 1 was finally by far the largest, while plant 2 had larger leaves than plants 3 and 4.

*E. anglica* (E76), collected on 25 May, was grown in two pots; one contained *Plantago lanceolata* and two grass species, but it became completely dominated by *Medicago lupulina* and produced two luxuriant *Euphrasia* plants as well as some smaller ones. The second pot, with many species, including *Medicago lupulina* but consisting mostly of grass, produced only moderately vigorous *Euphrasia* plants, similar to the weaker ones in the first pot. The pot containing *E. anglica* (E67), collected from the same place as (E76) on 15 April, became dominated by *Holcus lanatus*, and only one *Euphrasia* plant developed (belatedly) good-sized leaves. *E. nemorosa* (E74), collected on 8 May, was very prolific, producing in one pot one luxuriant plant, five vigorous ones, seven medium ones and four small ones; all had deep-green leaves. This pot had become
dominated by *Medicago lunulina*, and contained also *Anthyllis vulneraria*, *Hieracium pilosella*, *Bromus erectus*, *Pimpinella saxifraga*, *Festuca ovina*, *Linum catharticum* and *Dactylis glomerata*. *E. pseudokernerri* (E75), collected on 8 May, produced one luxuriant, deep-green plant and three smallish, belatedly established, pale-coloured plants plus one very weak one. There were two turves in the pot; one, with the luxuriant *Euphrasia* plant and one of the small ones, contained *Anthyllis vulneraria* (dominant), *Dactylis glomerata*, *Linum catharticum*, *Plantago media*, *P. lanceolata* and *Festuca ovina*; the other turf contained the remaining Euphrasias, and had grasses dominant among *Koeleria gracilis*, *Festuca* sp., *Lotus corniculatus*, *Thymus drucei*, *Anthyllis vulneraria*, *Plantago media* and *Poterium sanguisorba* (seedling). By contrast, *E. nemorosa* (E72), collected on 3 May, produced a few plants that were slender and small-leaved, though quite well grown, and several very weak ones; the pot contained no Leguminosae, but several other dicotyledonous herbs, several grasses and one *Carex*. Thus, in their luxuriant growth and dark-green leaves, the plants growing with vigorous Leguminous species show symptoms of a good supply of nitrogen, which they must get either through their haustoria or through the soil after its release by the legumes. The former hypothesis seems more probable, in view of the differences existing between individual Euphrasias in the same pot.

I continued to collect *Euphrasia* plants for cultivation
in 1952 until 13 September. Those collected from June to September were dug up in their turves and were not separated from them. Many species were collected from diverse habitats. In the great majority of samples collected in this way (including some sent through the post from N. Wales in June and from Somerset in August) some of the Euphrasias continued to grow and flower, though some temporarily stopped flowering shortly after being collected and some were attacked by disease.

Host Trial, 1953.

The Euphrasias used were *E. pseudokerneri* (E226) and *E. anglica* (E157). Four *Euphrasia* seedlings were planted in each pot, the pots being 3\(\frac{3}{4}\)-4 inches in diameter. For each species of *Euphrasia* there were two pots with each of 21 host species. There were two host-plants per pot, except in the case of *Achillea millefolium* where there was only one per pot. The hosts were of known origin and were in the form of young seedlings or portions of newly-divided plants. The pots were plunged in an ash-bed in a sheltered but sunny position at University College, Leicester.

The Euphrasias and hosts were planted out from 28 to 31 March. By 20 April many Euphrasias were dead and many damaged, apparently by frost. Replacements were made, but not all the pots could be brought up to their full numbers.

In addition to the pots with hosts, some pots were set up without hosts, as follows: for each species of *Euphrasia*
three clusters of seedlings were placed in each of two 4-inch pots, and a single seedling in each of five 3-inch pots; all these were also planted out at the end of March. The two seed-pots, containing the original sowings of *E. pseudokerner* and *E. anglica*, had been in the greenhouse for a time, and on 24 April they were plunged with the rest of the pots. The seed-pot of *E. pseudokerner* contained about 67 seedlings in a close group, and a few other more or less isolated ones; that of *E. anglica* had 8 seedlings left, grouped fairly closely.

Most of the plants were pressed on 20 September, but a few were pressed on 1 and 3 September. The appearance of some of the more vigorous plants at this time is shown by Fig. 32, p. 82. Most pots had roots of the host-plants coming out of their drainage-holes in September, and a few had *Euphrasia* roots emerging. In each species, one of the single plants with no host had roots emerging from the pot, and these plants were larger than the other single plants. It is possible that these roots could have parasitized weeds growing outside the pots.

In order to present the results as simply as possible, the *Euphrasias* have been graded into six sizes. A "type-plant" of *E. pseudokerner* was chosen for each size, and the other plants were then classified into the six grades. Silhouettes of the "type-plants" are shown in Figs. 33 & 34, pp. 83-84. Most of the *E. pseudokerner* plants were compact and bushy, but a few were rather drawn up, less
FIG. 33: Silhouettes of *E. pseudokernerii* (E226). "Type-plants" of grades 1 to 5, natural size. Right to Left: 1) no host, seed-pot; 2) no host, small pot; 3) host - *Agrostis gigantea*; 4) host - *Sieglingia decumbens*; 5) host - *Hieracium pilosella*. 
FIG. 34: Silhouette of *E. pseudokerneri* (E226). "Type-plant" of grade 6, natural size. Host - *Anthyllis vulneraria.*
profusely branched, and somewhat difficult to classify. The "type-plants" of E. pseudokerneri were also used for grading E. anglica, and difficulty owing to difference in habit was more frequent here. However, the grading in the difficult cases is probably reasonably accurate.

The performance of the Euphrasias according to this grading is given in Table 8, p. 86. Where Euphrasia plants in grades 5 or 6 (the largest plants) appear in the table the host was very good; the host was moderate to rather poor if the highest grade is 3 or 4; if the highest grade is 1 or 2 the host (or lack of host) gave very poor results. Owing to the small number of Euphrasias with each host species, however, it is inadvisable to conclude, from the results of this experiment, that any host is a poor one, though some are clearly good. Thus, in 1952 E. pseudokerneri grew well on Plantago lanceolata and on Carex caryophyllea, while on most other occasions E. anglica has grown well on Medicago lupulina. It was shown in a later experiment with E. nemorosa that some hosts, though they give good growth, are also liable to give a high proportion of failures to become established. This factor could be responsible for the variations of behaviour with the hosts just mentioned. It cannot be claimed with confidence that the two Euphrasia species differ in their host-reactions, but the results suggest that the best hosts for E. pseudokerneri are mostly dicotyledons, and that the best hosts for E. anglica are monocotyledons.
### TABLE 8: NUMBER OF EUPHRASIAS IN EACH GRADE, SEPTEMBER 1953

#### E. pseudokerneri (E226)

<table>
<thead>
<tr>
<th>Host</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<td>Dactylis glomerata</td>
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<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hieracium pilosella</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anthyllis vulneraria</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medicago lupulina</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus drucei</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Festuca ovina</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koeleria gracilis</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agrostis gigantea</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachypodium pinnatum</td>
<td>3</td>
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<td></td>
</tr>
<tr>
<td>Sieglingia decumbens</td>
<td>2</td>
<td>1</td>
<td>1</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>Achillea millefolium</td>
<td>1</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Carex flacca</td>
<td></td>
<td></td>
<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No host - seed-pot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Many</td>
</tr>
<tr>
<td>No host - large pot</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No host - small pots</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bromus erectus</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex demissa</td>
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<td>1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex caryophyllea</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex p. pilulifera</td>
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</tr>
<tr>
<td>Plantago lanceolata</td>
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#### E. anglica (E157)

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<td>Agrostis gigantea</td>
<td>2</td>
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<td></td>
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<tr>
<td>Luzula campestris</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Brachypodium pinnatum</td>
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<td></td>
</tr>
<tr>
<td>Carex caryophyllea</td>
<td>3</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Carex p. pilulifera</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Anthyllis vulneraria</td>
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<td>Medicago lupulina</td>
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<td>No host - seed-pot</td>
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</tr>
<tr>
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<td>2</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>No host - small pots</td>
<td>1</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>Bromus erectus</td>
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</tr>
<tr>
<td>Achillea millefolium</td>
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<tr>
<td>Carex flacca</td>
<td>2</td>
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<tr>
<td>Carex demissa</td>
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<tr>
<td>Thymus drucei</td>
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<td>Helianthemum</td>
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<tr>
<td><em>chamaecistus</em></td>
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</table>
In three pots, roots of *E. pseudokerneri*, as well as roots of the host-plants, had emerged from the drainage-holes by September, and they bore detached haustoria. The hosts concerned were *Thymus drucei*, *Hieracium pilosella* and *Koeleria gracilis*. Most probably the haustoria had been attached to the host-roots before the pots were moved. The Euphrasias in the pot with *Koeleria gracilis* were poorly developed. It thus seems probable that some hosts, at least, may be poor not because they prevent the formation of haustoria but because they fail to provide much of what the Euphrasias require even though haustoria are formed. However, the possibility exists in this case that the haustoria were attached to other Euphrasia roots.

Of the plants with no host, some of both species flowered in the seed-pots, in the small pots and in the large pots, even where no roots emerged from the pots. Very small capsules ripened on *E. anglica* in each type of pot, and on *E. pseudokerneri* in a small pot; seed of the latter species would probably have ripened also in the other two types of pot if the plants had been left longer before being pressed. The reason for planting clusters of Euphrasias without a host in the larger (4-inch) pots was to see whether, by parasitizing their fellows, some of them would grow more vigorously. In the two pots with *E. anglica* in clusters, only two survived in one pot and only one in the other. In the only remaining pot with *E. pseudokerneri* in clusters, three plants remained and one was larger and
more branched than the others; this was just dead but bore three withered flowers on 20 September, while one of the others was still alive and probably ripening one or two capsules. These few plants do not justify the drawing of any conclusions about auto-parasitism.

Use of Sagina as a Host-Plant.

Six *Euphrasia* forms were cultivated with *Sagina* seedlings obtained from pots in the greenhouse. One *Sagina* plant and four seedlings of a *Euphrasia* were placed in each of six pots, which were $3\frac{1}{2}$ inches in diameter and were kept in the greenhouse. The *Euphrasias* were as follows:

*E. micrantha* (E185A), *E. confusa* (E183C), *E. nemorosa* (E210), *E. anglica* (E180), *E. rostkoviana* (E233) and *Euphrasia* sp. (E231). The last named does not agree with any recognized species, but the details of its leaf-teeth suggest that it is derived from *E. micrantha*. The *Saginas* all proved to be *S. apetala*, except for the plant which was with *E. micrantha* which was *S. procumbens*.

The potting up was carried out on 24 April 1953. One plant of *E. rostkoviana* was dead by 15 May, and this was the only plant in the experiment that died. The plants were pressed on 6 or 7 August, with the exception of *E. micrantha* which was pressed on 3 September.

Apart from one plant of *Euphrasia* sp. (E231) and one of *E. anglica*, all the surviving plants appeared to become established on the host. The first signs of establishment
were seen on 15 May, only three weeks after potting up, in
E. micrantha, E. confusa and E. nemorosa, while symptoms of
it were seen in Euphrasia sp. (E231) on 23 May, and in
E. anglica and possibly E. rostkoviana on 1 June. Taking
into account the small size of the pots and the final
reasonably large stature of the plants, it seems certain
that establishment on the host did take place. E. rost­
koviana and E. anglica were very similar to one another in
vigour, and appeared less vigorous than the four other
species, for which Sagina is probably quite a good host.

The results are illustrated by photographs (Figs. 35-
39, pp.90-93), which incidentally demonstrate clearly the
retention in cultivation of the distinguishing characters
of the species.

The unestablished plants may have been parasitized by
their fellows: the one of E. anglica was about a quarter
the size of the smallest other plant in the pot, and that
of Euphrasia sp. (E231) perhaps one tenth the size of its
smallest companion and about half the size of the unestabli-
shed E. anglica plant.

Effect of Soil Variation on Euphrasias with no Host.

Seedlings of Euphrasia anglica (E240) were collected
on 28 February 1953. They were intended to be a reserve
for cultivation experiments. Three or four days after
collection, about 120 seedlings were removed from their
turf and planted in two pots (numbered 1 and 2) in approxi-
FIG. 35: *Euphrasia* sp. (E231) with *Sagina apetala*. 19 July 1953.
FIG. 36: *E. micrantha* (E185A) with *Sagina procumbens*. 19 July 1953.
FIG. 37: *E. nemorosa* (E210) with *Sagina apetala*. 2 August 1953.
FIG. 38: E. confusa (E183C) with Sagina apetala. 2 August 1953.

Fig. 39: Left - E. anglica (E180) with Sagina apetala. Right - E. rostkoviana (E233) with Sagina apetala. 19 July 1953.
mately equal numbers. About three days later a third pot (number 3) was set up with about 30 seedlings. The pots were kept in a greenhouse, the window of which was open almost continuously.

The plants were not needed for any other purpose, and grew on for some time. On 22 May pot 1 had 42 seedlings and pot 2 had 39. In pot 1 the two smallest plants were pale green throughout, and each had six pairs of leaves about 2mm. long and 1.5mm. broad, with only one tooth on each side. The rest of the plants in this pot were medium green, but paler at the top; the two largest had eight and nine pairs of leaves visible respectively, measuring about 5mm. by 3mm. and with up to two teeth on each side; one of them had branch initials. The majority were nearer the largest than the smallest in size. This also applied to pot 2, but the largest were slightly smaller than in pot 1, and the smallest were similar to the smallest in pot 1. All the plants in pot 2 were distinctly chlorotic, the upper leaves being quite yellowish. The leaves appeared slightly fleshy. A photograph of these two pots (Fig. 40, p.95) shows some of these differences. Pot 3 had twelve seedlings, similar to those of pot 1 and with a similar size range, but with a slightly smaller maximum size. They were medium green except for the upper leaves of some of the large ones, which were pale.

On 12 June two plants in pot 1 had their first flowers open, three more were ready to bloom shortly, and others had
flower buds. In pot 2 all the plants were chlorotic and no flower buds were visible. In pot 3 there were flowers nearly ready to open on two plants. The differences between pots 1 and 2 at this time are shown in Figs. 41 & 42, p. 96.

The poorer condition of the plants in pot 2 is believed to have been due to differences of soil. The soil in pot 2 appeared lighter in colour and with less peat at the surface than that in pot 1; and, unlike the soil in pots 1 and 3, it had mosses and green algae developing on it by 22 May. Pot 3 appeared to have yet more peat than pot 1, and also more loam and less sand.

FIG. 41: *E. anglica* (E240), pots 1, mid-June 1953.

FIG. 40: *E. anglica* (E240), pots 1 and 2 on 22 May 1953. The plants in pot 2 (Right) are chlorotic.
Cultivation of Three Euphrasia Species with Four Host Species.

In this experiment, E. anglica (E240) was grown indoors and outside in pots: E. accidentalis (E240), E. hesperis (E240), E. anglica (E240), Medicago sativa (E240), and Trifolium dubium (E240). The aim was to test the Euphrasias' ability to colonize the soil and the presence of a host varied greatly. The experiment consisted of six host seedlings of Euphrasias were planted towards the edge, but the exact positions of the pots, each pot being provisioned for host seedling, were varied. The hosts consisted of dead Euphrasias until 30 May. The pots were then replaced by new seedlings. The plants emerged through gaps at the base of the pots, and the roots formed a new, and the pots were placed on a shelf apart. The roots of the plants were then observed. The experiment concluded.
Cultivation of Three Euphrasia Species with Four Host Species.

In this experiment *Euphrasia borealis* (E329) was grown indoors and outdoors, and *E. nemorosa* (E417) and *E. occidentalis* (E351) were grown indoors only.

*Medicago lupulina* (= M), *Plantago lanceolata* (= P) and *Trifolium dubium* (= T) were used as host-plants for the Euphrasias indoors. There was one pan of each Euphrasia species with each host, except that there was an extra pan of *E. occidentalis* with *Medicago lupulina*. The potting-up and the pressing of the plants was done on a number of dates, so that the period of growth in the presence of a host varied from pan to pan. In each pan four seedlings of the host-plant were placed near the centre, and eight seedlings of *Euphrasia* were planted towards the edge, but pot 2 of *E. occidentalis* with *Medicago* contained six host seedlings. Six Euphrasias died and were replaced in the first three weeks.

*E. borealis* was grown outdoors with the same three hosts and with *Briza media* (= B). The Euphrasias were planted singly on 11 May in bituminized paper pots, each pot being provided at the same time with one host seedling. There were ten pots with each host species. Replacements of dead Euphrasias were made from time to time until 30 May. On 2 June the pots were embedded in the soil of the experimental ground; the pots with each host formed a row, and the rows were placed two feet apart. The roots of the plants emerge through gaps at the base of the pots, and
through holes which appear as the pots rot.

During development the host plants outdoors were cut back where necessary to stop them overshadowing the Euphrasias. On 11 July observations were made on the state of the plants, and on 3 September all but four very small ones were pressed.

To compare the growth of the plants, the sum of the lengths of stem and branches of each pressed plant was calculated. The branching of E. nemorosa with Plantago was too complicated for direct measurement, and the total length was estimated by measuring selected branches and counting the number of branches of approximately the same length. The total length of stem and branches gives a fairly good indication of vigour, but leaf-size is also important.

The results are given in Table 9 (p. 99), which shows that the average size of E. nemorosa plants was three times as great with P as with M, and that the average size of E. borealis plants indoors was twice as great with P as with M. But since more Euphrasias died with P it may be more appropriate to compare the total length of Euphrasia stems and branches produced in each pot. When this is done (right-hand half of Table 9), P is seen to have produced twice as much Euphrasia nemorosa as M, and so still appears superior although the growing period for P was 10 days shorter than for M. With E. borealis, however, the former apparent superiority of P is almost eliminated, but the
TABLE 9: TOTAL LENGTH MEASUREMENTS OF EUPHRASIA PLANTS, 1954

<table>
<thead>
<tr>
<th>Euphrasia</th>
<th>Average (in cm.)</th>
<th>Aggregate for each pot (in cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>P</td>
</tr>
<tr>
<td>occidentalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pot 1)</td>
<td>11 (7 plants)</td>
<td>7.5(4)</td>
</tr>
<tr>
<td></td>
<td>&quot; (pot 2)</td>
<td>-</td>
</tr>
<tr>
<td>nemorosa</td>
<td>65 (7 &quot; )</td>
<td>182(5)</td>
</tr>
<tr>
<td>borealis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(indoors)</td>
<td>15 (8 &quot; )</td>
<td>28(5)</td>
</tr>
<tr>
<td>&quot; (outdoors)</td>
<td>10 (4 &quot; )</td>
<td>41(5)</td>
</tr>
</tbody>
</table>

Growing period of *E. borealis* with M was 21 days shorter than with P, which suggests that M may in fact be superior to P for this *Euphrasia*. However, the pressed plants show that only two or three of the eight plants with M would have grown much after the date of pressing, 23 July. The development of *E. occidentalis* was, on the average and in the aggregate, better with M (in pot 1) than with P, but it was poor on both these hosts. Very much better growth resulted with M in pot 2. This pot, however, had been set up much earlier, and the Euprasias gained a considerable advantage from this, becoming established on the host eight weeks earlier than those in pot 1. In pot 2, leaf size was also much larger than in pot 1. *T* is seen to be a very inferior host for all the Euprasias grown with it; the death of all the indoor plants of *E. borealis* with *T* is
probably accounted for by the unsatisfactoriness of this host.

The indoor cultivation can be summed up by saying that M and P were good hosts for *E. nemorosa* and *E. borealis*, M was fairly good for *E. occidentalis*, and T was very poor for all the Euphrasias. It will be noted that more Euphrasias of all species survived with M than with P.

Outdoors, B was a failure for *E. borealis*, T was a virtual failure, M was poor, and P was very good at least as regards vigour although perhaps not as regards survival (Table 9, p.99). Four out of five outdoor plants of *E. borealis* with P were very vigorous with large or very large leaves. A sixth plant was also vigorous, but only a fragment of it was alive at the time of pressing, so that it could not be measured. Whereas *E. borealis* grew better outdoors than in on P, on M it grew better indoors than out.

The observations that were made on 11 July on the plants of *E. borealis* in this trial are given in Table 10, p.101. Here, as in Table 9, P appears as the best host for *E. borealis* outdoors, M is again second best, and T and B are both very poor.

This experiment provided some further information on the time taken for establishment on the host, which was judged in this case by the general appearance of the plants. The times taken are given in Table 11, p.101. No observations were made between 15 June and 11 July, and this
### TABLE 10: OBSERVATIONS ON E. BOREALIS OUTDOORS, 11 JULY 1954

<table>
<thead>
<tr>
<th>Host</th>
<th>Trifolium dubium</th>
<th>Plantago lanceolata</th>
<th>Briza media</th>
<th>Medicago lupulina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number alive</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Number apparently host-established</td>
<td>0</td>
<td>8 or 9</td>
<td>0</td>
<td>3 possibly</td>
</tr>
<tr>
<td>Number flowering</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

### TABLE 11: TIME TAKEN FOR ESTABLISHMENT OF EUPHRASIAS ON HOSTS

<table>
<thead>
<tr>
<th>Euphrasia</th>
<th>Host</th>
<th>Establishment period</th>
</tr>
</thead>
<tbody>
<tr>
<td>occidentalis</td>
<td>M (pot 1)</td>
<td>62 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>M (pot 2)</td>
<td>74 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>P</td>
<td>62 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>T</td>
<td>-</td>
</tr>
<tr>
<td>nemorosa</td>
<td>M</td>
<td>30 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>P</td>
<td>36-62 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>T</td>
<td>36 days</td>
</tr>
<tr>
<td>borealis</td>
<td>M indoors</td>
<td>26 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>P</td>
<td>36-62 days</td>
</tr>
<tr>
<td>&quot;</td>
<td>T</td>
<td>-</td>
</tr>
<tr>
<td>&quot;</td>
<td>M, P, T &amp; B outdoors</td>
<td>29-61 days</td>
</tr>
</tbody>
</table>
explains the maximum of 62 days given for some samples; the
greatest time required was probably less than 62 days.
These figures may be compared with the times of 72 to 95
days for *E. pseudokerneri* grown from seed in 1952, 40 to 50
days for wild seedlings of the same species in 1952, and 21
to 38 days for the *Euphrasia* species grown from seed with*Sagina* in 1953.

**Growth of Euphrasia with Various Host Species, 1953 & 1954.**

Many species of *Euphrasia* were grown in pots in 1953
and 1954, and a number of host species were used. Some of
the pots were kept in the greenhouse, and others were
plunged in ash outdoors. The following list describes the
growth of the Euphrasias on the hosts named.

**Ranunculus repens with:**

*E. anglica* (E139) - poor to moderate

**Medicago lupulina with:**

*E. micrantha* (E185A) - moderate to vigorous
*E. occidentalis* (E192) - moderate
*E. nemorosa* (E166) - luxuriant
  " (E211) - very luxuriant
  " (E419) - vigorous
*E. confusa* (E172) - moderate to luxuriant
  " (E208) - poor to luxuriant
  " (E406) - vigorous
*E. pseudokerneri* (E70) - vigorous
*E. brevipila* (E325) - poor
  " (E426) - poor
  " var. reayensis (E308) - poor
*E. suecica* (E424) - vigorous
*E. anglica* (E180) - vigorous
  " (E71) - vigorous
  " (E168) - moderate to vigorous
  " (E135) - moderate to vigorous
*E. vigursii* (E348) - moderate
*E. salisburgensis* (E204) - vigorous to luxuriant
  " var. hibernica (E230) - moderate to luxuriant
Anthyllis vulneraria with:

- E. foulaensis (E227) - vigorous
- E. curta var. rupestris (E100) - poor to moderate
- E. occidentalis (E192) - poor to moderate
- E. confusa (E183C) - moderate to vigorous
- E. pseudokerneri (E226) - luxuriant

Calluna vulgaris with:

- E. micrantha (E185A) - poor
  " (E112A) - vigorous

Calluna vulgaris and Erica cinerea (mixed) with:

- E. micrantha (E185A) - vigorous

Thymus drucei with:

- E. occidentalis (E192) - poor to moderate
- E. salisburgensis (E204) - vigorous

Plantago lanceolata with:

- E. occidentalis (E192) - moderate to vigorous

Luzula campestris with:

- E. micrantha (E185A) - very poor
- E. rivularis (E123) - moderate
- E. rostkoviana (E233) - poor
  " (E239) - vigorous
  " (E234) - poor
  " (E236) - poor to moderate
  " (E237) - poor to moderate

Carex pilulifera with:

- E. micrantha (E185A) - very poor

Carex carvophyllea with:

- E. confusa (E174) - very poor
  " (E208) - poor to moderate
Sieglingia decumbens with:

E. occidentalis (E192) - poor to moderate
E. nemorosa (E196) - moderate

Bromus erectus with:

E. occidentalis (E192) - poor to moderate

Koeleria gracilis with:

E. cf. micrantha (E231) - poor
?E. confusa x micrantha (E107) - very poor

Agrostis tenuis with:

?E. confusa x micrantha (E107) - poor
E. occidentalis (E192) - poor to moderate
E. nemorosa (E196) - moderate

FIG. 43: *E. salisburgensis* var. *hibernica* (E230), growing in a pot 5 inches in diameter with *Medicago lupulina*. 1 June 1953.
Carex caryophyllea was used as host in seven pots of E. confusa in addition to those listed above. There were 10 Euphrasia seedlings per pot, and those in each pot were drawn from a different population. No plants worth pressing survived. With Koeleria gracilis a further nine pots, each with ten Euphrasias representing populations of E. nemorosa and probably E. confusa x micrantha, also produced no plants worth pressing. Leontodon autumnalis, which does not appear in the above list, was also tried thoroughly and all the Euphrasias with it died. The following Euphrasias were tried with it: E. foulaensis - 10 seedlings; E. occidentalis - 10 seedlings of each of two populations; and E. anglica and hybrids of E. anglica - 14 seedlings of each of 11 populations.

Good growth was frequently obtained both in the greenhouse and in the pots plunged outdoors, and it is not considered that this difference of situation had any pronounced effect on the behaviour of the Euphrasias.

It is noteworthy that E. micrantha grew well on Medicago lunulina as well as Ericaceae, though in nature it is rarely found apart from Ericaceae. Both the Leguminosae used as hosts were usually good (Fig. 43, p.104, and Fig. 44, p.106). Luzula campestris was quite good for E. rivularis and, in one instance, for E. rostkoviana (Fig. 45, p.107). The grasses varied, but none was very good, and Koeleria gracilis was particularly poor. Leontodon autumnalis was a complete failure, in strong contrast to Hieracium pilosella,
a Composite which had been a good host in experiments described earlier. The plants described above were not examined for haustoria, but I consider it safe to assume that all those described as moderate, or better, were benefitting parasitically from their hosts. Those described as poor or very poor were growing no better than they might have done in the absence of a host. The cultivated plants of these species, except E. rivularis, retained the characters which they show in the wild; E. rivularis became much more vigorous, more robust-looking and larger leaved (Fig. 45, p.107).

Twelve samples of the 1954 plants in the above list took 26 to 55 days to become established on the host. In seven of these samples, all the plants became established 30-45 days after being potted up. In addition, some plants in a few other samples took longer than 55 days, but the exact time is not known owing to a gap in recording from 15 June to 23 July. The host for all these plants was Medicago lunulina.

In some instances there were two or more pots of about the same size with the same Euphrasia and the same host-species, but with varying numbers of Euphrasia plants. The sum of the lengths of stem and branches of the pressed Euphrasias grown in these pots was calculated, and the totals for all the plants in each pot are given in Table 12, p.109. This shows that more growth in the aggregate can be obtained by increasing the number of plants per pot, but
TABLE 12: GROWTH OF EUPHRASIA PLANTS COMPARED WITH NUMBER PER POT

<table>
<thead>
<tr>
<th>Euphrasia species</th>
<th>Host</th>
<th>No. of Euphrasias in pot</th>
<th>Aggregate length per pot</th>
<th>Mean length of plants</th>
<th>Pot diameter in inches</th>
</tr>
</thead>
<tbody>
<tr>
<td>micrantha (E185A)</td>
<td>Medicago lumulina</td>
<td>4</td>
<td>97 cm.</td>
<td>24 cm.</td>
<td>3 1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>142</td>
<td>24</td>
<td>3 1/2</td>
</tr>
<tr>
<td>occidentalis (E192)</td>
<td>Plantago lanceolata</td>
<td>2</td>
<td>45</td>
<td>23</td>
<td>3 1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>47</td>
<td>16</td>
<td>3 1/2</td>
</tr>
<tr>
<td>occidentalis (E351)</td>
<td>No Host</td>
<td>5</td>
<td>32</td>
<td>6</td>
<td>2 3/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>28</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>30</td>
<td>4</td>
<td>3 1/2 (or 2 3/4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>58</td>
<td>4</td>
<td>3 1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>74</td>
<td>4</td>
<td>3 1/2 (or 2 3/4)</td>
</tr>
<tr>
<td>anglica (E135)</td>
<td>Medicago lumulina</td>
<td>1</td>
<td>38</td>
<td>38</td>
<td>3 1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>37</td>
<td>37</td>
<td>3 1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>65</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>63</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>58</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>rostkoviana (E233)</td>
<td>Luzula campestris</td>
<td>6</td>
<td>66</td>
<td>11</td>
<td>5 1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>138</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

there is then some reduction in plant-size, as indicated by the slight downward trend of mean length with increasing number of plants per pot.


The following notes describe the pressed Euphrasias which had been grown without host-plants in these years. The pots were 5 1/2 inches in diameter unless otherwise stated.

E. micrantha (E185A). Pressed 2/9/53. One pot. Fairly numerous. 2-3(-4) cm. tall.
Not flowering: few.
Flowering or having flowered: a fair number.
Fruiting: rather few (seems to flower late).
With branches flowering: few.
E. occidentalis (E192). Pressed 2/9/53. Pot (1). Rather numerous. Mostly .6-1.5cm. tall (internodes very short).

Not flowering: few.
Flowering: most plants.
Fruiting: many.
Branching: possibly a few.

Pot (2). Very numerous. Up to 1.5cm. tall.
Not flowering: many (5mm. or less tall).
Flowering: many.
Fruiting: many.
Two plants had noticeably larger and greener leaves than the rest.

E. occidentalis (E351). Pressed 9/7/54. Five pots, 2\ 1/4 or 3\ 1/2 inches in diameter. 2-9cm. tall. (See Fig. 46, p.111).

Unbranched: all.
Not flowering: 5 (all diseased) in a pot containing 18 plants.
Flowering and/or fruiting: 13 in pot containing 18 plants; all in 4 pots each containing 5-15 plants.

Extremely numerous. 1-3(-5)cm. tall.
Not flowering: many.
Flowering or having flowered: very many.
Fruiting: a fair number.
With branches flowering: a few.

Not flowering: 3 plants, 1-2cm. tall, branching.
Having flowered: 1 plant, 4cm. tall, branching.

Pot (2), 2\ 3/4 inches in diameter. Nine plants.
Not flowering: 5 plants (one .8cm. tall, unbranched; one 2cm. tall, branched; three dead).
Having flowered but not fruiting: 1 plant, 2cm. tall, branched.
Fruiting: 3 plants, 2cm. tall, branched.

E. nemorosa (E417). Pressed 27/8/54. One pot, 2\ 3/4 inches in diameter. 15 plants. 1.7 - 3.5cm. tall.
Not flowering: 11 plants, unbranched (8 dead, or nearly dead).
Flowering: 4 plants, unbranched (not permitted to fruit).
FIG. 46: *E. occidentalis* (E351) flowering without a host. About 14 June 1954.
E. confusa (E172). Pressed 2/9/53. One pot. Rather numerous. .7 - 3.5cm. tall.
Flowering: many.
Fruiting: a fair number.
Branching: a fair number (up to 3 branching nodes per plant).
With branches flowering and/or fruiting: a few.

.6 - 3.6cm. tall.
Not flowering: a few, excluding fruiting plants.
Flowering: a few.
Fruiting: many.
Branching: a few (up to 6 branching nodes per plant).
With branches fruiting: two.

E. brevipila (E325). Pressed 9/7/54. One pot, 2½ inches in diameter. Six plants.
Not flowering: 1 plant, .7 cm. tall.
Fruiting: 5 plants, 2-3cm. tall.

E. brevipila var. reavensis (E308). Pressed 24/7/54. One pot, 2½ inches in diameter. Six plants. Average height 6.8cm.
Flowering or with flower-buds: all plants (not permitted to fruit).

14 plants, 2 - 6.5cm. tall; 2 plants, 1.2cm. tall.
(See Fig. 47, p.113).
Unbranched: all.
Not flowering: the 2 small plants.
Flowering and/or fruiting: the 14 larger plants, 5 of them having died after ripening their seed.

Unbranched: all.
Not flowering: several (some of them dead, or dying).
Flowering: a few.
Fruiting: many (some dead).

It will be seen that all seven species of Eunhrasia in the list flowered and fruited without a host, there being
never fewer than four plants in a pot. Under these conditions, those species which are normally the most branched frequently produced branches, while the less branched species failed to produce any. Some species were more affected than others by the lack of a host; thus *E. nemorosa* (E421 and E417) grew much less well than *E. occidentalis* (E351), although the conditions of growth were similar. In pot (2) of *E. occidentalis* (E192) the plants were poorer than in pot (1) probably because of their excessive number. The two larger and greener plants in pot (2) of this sample looked as if they might have been parasitizing their fellows.
A Host Trial with Euphrasia nemorosa (E507) in 1955.

The object of this experiment was to grow, for purposes of comparison, Euphrasia nemorosa with four host species and with no host, varying the numbers of Euphrasias and host-plants per pot.

With no host the following pots were prepared:

6 3½-inch pots (NH) with 1 Euphrasia each,
6 5½-inch " (NH1) with 1 Euphrasia each,
4 " " (NH3a) with 3 Euphrasias each, spaced evenly,
4 " " (NH3b) " 3 " " , close together,
2 " " (NH6) " 6 " " , spaced evenly.

A 5½-inch diameter pot holds 2½ times as much soil as a 3½-inch one.

The four host species were:

Hieracium pilosella (= H),
Plantago lanceolata (= P),
Bromus erectus (= B),
Medicago lumulina (= M).

The following 5½-inch pots were prepared for each host species:

6 with 1 host-plant and 1 Euphrasia each, labelled H1, P1, B1, M1,
4 " 1 " " 3 Euphrasias each, labelled H3, etc.,
2 " 1 " " 6 " " " H6a, " ,
2 " 3 " " 6 " " " H6b, " ,

The Euphrasias and hosts were potted up as young seedlings. The Euphrasias were placed 1 to 1½ inches from
the edges of the pots, and the hosts were placed in the middle, except when only one *Euphrasia* and one host were present, in which case the *Euphrasia* and the host were both 1 to 1\(\frac{1}{2}\) inches from the edge of the pot but diametrically opposite. The distance between the Euphrasias and their hosts was, therefore, greatest in H\(_1\) etc. and least in H\(_6\) etc. During growth, the host plants were cut back where they tended to shade Euphrasias.

The potting up was done on 8 and 9 April. Some replacements were already necessary on 10 April, and further deaths were observed on 18 April and 27 April. Because the seedlings in the seed-pot were crowded and suffered from damping-off, insufficient replacements were available, and Euphrasias were removed from some of the depleted pots to complete others. In this way the number of pots was reduced below what had been intended. Mortality continued throughout the period of cultivation, but no more removals were made after 27 April, so that many pots eventually had fewer than the intended number of Euphrasias.

Observations were made on all plants about once a week from 8 May to 4 July. On 13 August a note was made of the survival or death of the plants, and the following day all were pressed.

*Sspeed of Establishment on the Host.*

A complicated graphical method of estimating when Euphrasias become established, based on number of leaf-teeth,
was described on p.60. In the present experiment, the
diameter of the seedlings (the greatest horizontal spread
of any pair of leaves on the main stem) was measured to
see whether it would afford a method of recognizing
establishment on the host. It was not, however, particularly
satisfactory because the upward slope of some leaves tended
to vary. I therefore timed the establishment of the
Euphrasias on their hosts by observing the sudden spurt of
growth, accompanied by a greener appearance of the new
leaves, which I have come to believe indicates establishment.

While some Euphrasias remained in their pots from
8 or 9 April, others were moved as late as 27 April. A few
plants were established by 8 May; assuming they had not
been moved, this indicates a time of 29 or 30 days for
establishment. The last plants to become established did
so by 21 June; if they had not been moved, the time taken
would have been 73 days; if they had been moved, it must
have been at least 55 days.

The graphs showing the dates of establishment can now
be considered (Figs. 48 & 49, pp.117-118). They show the
percentage of Euphrasias established in each of the first
six weeks of the observation period; no establishments were
observed in the remaining two weeks. The number of plants
alive at the beginning and end of the six weeks is shown.
There was at this time little departure from the intended
numbers of plants per pot. There is little variation in
time of establishment on the different hosts (Fig. 48).
FIG. 48: Establishment of Euphrasias on host-plants, shown for different species of host.
FIG. 49: Establishment of Euphrasias on host-plants, shown for different numbers of plants per pot.
The total percentage of *Euphrasia* plants that became established did, however, vary according to host, H and M giving the best results - 73% and 76% respectively, compared with 64% for both P and B. Comparing numbers of plants per pot (Fig. 49), it will be seen that establishment on the host was quickest with six *Euphrasias* and three host-plants per pot, where the distance between host and *Euphrasia* was least. Establishment was slowest with one *Euphrasia* and one host-plant per pot; this was the class where the distance between *Euphrasia* and host was greatest. However, the two remaining classes, with one host, and either three or six *Euphrasias*, though they did not differ from one another in the distance between the host and the parasite, differed in speed of establishment, the *Euphrasias* which were only three to a pot becoming established sooner. The total percentage of plants becoming established in the four classes varied in some degree as speed of establishment, the class with one *Euphrasia* and one host per pot giving a considerably lower percentage than the rest.

The percentages of *Euphrasias* transplanted on 18 and 27 April, near the beginning of the experiment, are given in Table 13, p.120. It will be seen that the percentage moved was greatest with the hosts P and M and with the class of three *Euphrasias* per pot. These removals may have affected the speed of establishment on the host, and the number of plants becoming established, but the results
TABLE 13: EUPHRASIAS TRANSPLANTED ON 18 AND 27 APRIL 1955
EXPRESSED AS A PERCENTAGE OF THOSE PRESENT ON 8 MAY 1955

<table>
<thead>
<tr>
<th>Host</th>
<th>Number of Euphrasias per Pot</th>
<th>1</th>
<th>3</th>
<th>6 with 1 host</th>
<th>6 with 3 hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>30</td>
<td>16</td>
<td>24</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>P</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>33</td>
<td>36</td>
<td>46</td>
<td>30</td>
<td>27</td>
</tr>
</tbody>
</table>

No. of Euphrasias present 8/5/1955
Percentage moved

given in Figs. 48 & 49, pp.117-118, show no effects that can clearly be ascribed to late transplanting.

**Final Weights of Euphrasias.**

After 21 June the mortality of the Euphrasias became rather severe. The plants were, therefore, reclassified according to the number present per pot on 14 August (the date when they were pressed). Several plants so reclassified had been in pots with a larger number of established plants for an appreciable part of their growth period. The pressed Euphrasias were weighed to the nearest 0.1gm.

A treatment applied in the NH class (p.114) was not applied to the other groups and will be dealt with first. Single Euphrasias were grown in small pots (NH) as well as in large pots (NH1). The weights of the plants were as follows - NH (3 plants): 0.2, 0.2 and 0.1gm.; NH1 (6 plants including 1 depleted pot of NH3b): 0.1, 0.1, 0.2, 0.1, 0.2 and 0.2gm. These few data indicate no improvement in growth when 2½ times as much soil per plant is available.
The pots that were reclassified on 14 August contained from one to six Euphrasias each. For a comparison between hosts and between different numbers of plants per pot, the pots were grouped according to whether they contained 1 or 2 Euphrasias, 3 or 4 Euphrasias, or 5 or 6 Euphrasias. For comparison between results with hosts and results without hosts, the NH group with small pots was excluded.

It will be seen from Table 14 that, when a host was present, there was no correlation between the average weight of the Euphrasia plants and the number per pot. With P as host the low average weight with five Euphrasias

<table>
<thead>
<tr>
<th>Number of plants per pot</th>
<th>Euphrasias</th>
<th>Hosts</th>
<th>H</th>
<th>P</th>
<th>B</th>
<th>M</th>
<th>NH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or 2</td>
<td>1 or 3</td>
<td>.33 (8)</td>
<td>.48 (4)</td>
<td>.07 (3)</td>
<td>.23 (4)</td>
<td>.15 (12)</td>
<td></td>
</tr>
<tr>
<td>3 or 4</td>
<td>1 or 3</td>
<td>.41 (10)</td>
<td>.49 (8)</td>
<td>.08 (4)</td>
<td>.28 (14)</td>
<td>.14 (10)</td>
<td></td>
</tr>
<tr>
<td>5 or 6</td>
<td>1 or 3</td>
<td>.32 (5)</td>
<td>.17 (5)</td>
<td>.13 (6)</td>
<td>.25 (11)</td>
<td>.08 (6)</td>
<td></td>
</tr>
<tr>
<td>1 to 6</td>
<td>1 or 3</td>
<td>.36 (23)</td>
<td>.39 (17)</td>
<td>.10 (13)</td>
<td>.26 (29)</td>
<td>.13 (28)</td>
<td></td>
</tr>
<tr>
<td>3 or 4</td>
<td>1</td>
<td>.40 (7)</td>
<td>.45 (4)</td>
<td>.08 (4)</td>
<td>.25 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 or 4</td>
<td>3</td>
<td>.43 (3)</td>
<td>.53 (4)</td>
<td>-</td>
<td>.35 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 or 6</td>
<td>1</td>
<td>.32 (5)</td>
<td>-</td>
<td>.13 (6)</td>
<td>.24 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 or 6</td>
<td>3</td>
<td>-</td>
<td>.17 (5)</td>
<td>-</td>
<td>.27 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 14: MEAN WEIGHTS (in gm.) OF EUPHRASIAS AFTER PRESSING
(The number of plants in each class is given in brackets).
per pot was probably due to chance, because of the great variation in size of the Euphrasias with this host. Since the number of Euphrasias per pot appears to make no difference to the weight of individual plants, the overall mean weight with each host species can be used for a comparison of hosts. These overall means are given in the fourth line of Table 14; they show that H and P gave high mean weights, M a fairly high mean, and B a slightly smaller mean than NH. The lower part of the table allows comparisons to be made between pots with one host plant and pots with three. There is some indication that a higher weight is obtained with three host plants than with one, but this may be due to the longer interval since host-establishment, as it has already been shown that establishment was more rapid when three hosts were present.

There was little variation in the weight of the *Euphrasia* plants with no host, and the relatively low weight with six Euphrasias per pot suggests that the quantity of soil was a limiting factor in growth. It seems probable that the roots of the host plants are much more efficient exploiters of the soil than *Euphrasia* roots, so that if a *Euphrasia* is in the same pot as a fully autotrophic plant from which it receives no benefit by parasitism it will be at a severe disadvantage compared with one alone in a similar pot. Therefore (as suggested on p.68) a complete failure of parasitism would be expected to lead to much worse development than the mere absence of a host.
Bromus erectus is clearly a very poor host for this population of Euphrasia nemorosa, but it seems very likely that the Euphrasia was in fact behaving parasitically and getting some benefit in this way. This view is supported by the fact that fourteen out of the twenty-two Euphrasias appeared to become established on this host in the period 8 May to 21 June. Evidently, subsequent growth was not nearly as good as with other hosts.

The variation found among individual plants with each host and with no host is shown in Fig. 50, p.124. It will be seen that H and P, which gave the highest average weights, also had the greatest range; the range of M is intermediate, and the ranges of B and NH are smallest. In order to give some idea of the appearance of the plants grown in this experiment, silhouettes of two herbarium specimens are given in Fig. 51, p.125.

Mortality.

Table 15, p.126, shows that low mortality of the Euphrasias occurred with the hosts M and H, as compared with P and B. The first two showed about 75% establishment between 8 May and 4 July, and the second two about 65% (Fig. 48, p.117). This bears out an impression obtained during my work on Euphrasia that plants which are not established are more liable to die than those which are. However, the very low mortality with no host (none between 4 July and 13 August) shows that this does not apply when
FIG. 50: Weight in gm. of pressed plants of *E. nemorosa*, shown for different hosts and no host.
FIG. 51: Plants of *E. nemorosa*, pressed 14 August 1955. Natural size. Left - One of two plants grown in a pot with no host; weight 0.2gm. Right - One of three plants grown in a pot with one plant of *Medicago lupulina*; weight 0.7gm.
TABLE 15: MORTALITY OF EUFRASIAS

<table>
<thead>
<tr>
<th>Class</th>
<th>No. alive on 8/5/55</th>
<th>Percentage of those alive on 8/5/55 dead by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4/7/55</td>
</tr>
<tr>
<td>H</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>P</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>41</td>
</tr>
<tr>
<td>M</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>NH</td>
<td>37</td>
<td>16</td>
</tr>
</tbody>
</table>

no host-plants are present. This bears out the theory that, if Euphrasias are in the same pot as a fully autotrophic plant from which they receive no benefit by parasitism, they will be at a severe disadvantage compared with Euphrasias in a pot by themselves.

Growth in the absence of a Host.

The growth of Euphrasias with no host in this experiment was better than in any of my previous experiments. One of the larger specimens grown with no host is shown in Fig. 51, p.125; it is 17cm. tall, whereas the previous maximum height recorded with no host was 9cm. for E. occidentalis (E351, see p.110).

Plants without hosts differed from plants with hosts in their fewer and shorter branches, which appeared to have begun developing late. The gradual increase in leaf-size gave the impression that the nourishment was improving slowly during the period of growth of these Euphrasias.
The majority of all the plants grown in this experiment, whether with a host or without, had begun to flower by 14 August, the date when they were pressed, and the rest showed indications that they would have flowered if they had been allowed to go on growing. The majority of the plants that had begun to flower had ripened some capsules, and this also applied equally to plants with hosts and to those without.

Colour.

It was noted on 13 August that Euphrasias growing on Medicago were almost invariably darker green than all the others. Similar observations were described on pp. 77-79, where it was suggested that Leguminous hosts provide the Euphrasias with an especially good supply of nitrogen.

Discussion.

The main ways in which the hosts varied are shown in Table 16. There was no variation in the speed of establish-

<table>
<thead>
<tr>
<th>TABLE 16: VARIATION OF EUPHRASIA ACCORDING TO HOST SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage established 8 May - 21 June</td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>73%</td>
</tr>
<tr>
<td>Average wt. of plants (gm.)</td>
</tr>
<tr>
<td>Weight range (gm.) up to</td>
</tr>
<tr>
<td>Mortality, 8 May - 13 Aug.</td>
</tr>
</tbody>
</table>
ment on the different hosts.

For experimental work it is desirable to have a high percentage of plants becoming established, a high average weight, little variation in weight, and low mortality. Euphrasias with B and NH show little variation in weight, but both are unsatisfactory in their low average weight, as is B in its high mortality. P has the highest average weight, but its establishment percentage is relatively low and its mortality high. H and M present the best combinations of characters. Since plants weighing 0.2gm. and upwards are quite well developed, M's lower average weight than H's is not a serious disadvantage, and it is counterbalanced by a smaller variation in weight and by lower mortality. The experiment, therefore, shows that M is the most useful host plant of those tested, and that three host plants per pot are better than one because establishment is quicker with three. In fact, since 1952, my first year of Euphrasia cultivation, I have used Medicago lupulina for nearly all indoor cultivations except host trials.

Cultivation of Euphrasia in the Garden.

In 1952 Euphrasia seedlings for cultivation in the garden were collected in spring from natural habitats. The species concerned were E. pseudokerneri (E70) and E. occidentalis (E73). A bed at Leicester was planted between 23 and 27 April 1952 with Plantago lanceolata, using plants grown from seed, or portions of plants dug up at Leicester;
they were spaced 4½ inches apart in rows of 36 individuals. Six rows, one foot apart, were prepared.

Seedlings of *E. pseudokerneri* were collected on 29 April, and three rows of 37 plants each were planted out between the Plantains on 29 and 30 April; on 1 and 5 May some dead Euphrasias were replaced. Two rows of *E. occidentalis* were planted out between the Plantains on 6 and 7 May, and some replacements were made soon afterwards. Very hot weather then set in and it seemed hopeless to try to save any Euphrasias. However, 22 plants of *E. pseudokerneri* survived until 20 June, 16 until 10 August, and 11 until 17 September, when they were pressed. Four of these were in a row which was covered by cloches from 20 June to 6 August. They were very small when pressed, as were those of one of the other rows; the third row had two very small plants and two that had apparently become established on the host. One of these latter had been noticeably well-developed on 6 August and had finished flowering by 17 September; it was about as vigorous as the more vigorous plants of *E. pseudokerneri* (E70) grown in the greenhouse in 1952, but it had relatively large leaves.

The other was quite small, but its last few pairs of leaves had increased considerably in size; presumably it had become established very belatedly.

All the plants of *E. occidentalis* were dead by 8 June; it may be recalled in this connection that plants of this population also failed to become established on hosts.
in the greenhouse after being separated from their native turf.

In 1953 a method using "whalehide" (bituminized paper) pots was used. One *Euphrasia* seedling and one host-plant were placed well apart in each pot of 3 or 3½ inches diameter, in a prepared compost. By using "whalehide" pots a suitably fine and lump-free soil was provided in which the delicate *Euphrasia* seedlings could be planted out. The main experiment included six seed samples grown for comparison at Leicester, five of which were *E. nemorosa* (E151, E166, E210, E211, E215), while the other was considered to be *E. stricta* (E167). The host plants were seedlings of *Plantago lanceolata*. The *Euphrasia* were grown from seed, and in all samples germination began in the second half of February or early in March. The potting up was done between 17 and 23 March, and the *Plantago* seedlings were planted a few days later; many replacements of *Euphrasia* had to be made on 23 and 24 March, and 84 pots of each sample were prepared. The "whalehide" pots were plunged in the experimental plot on 31 March to 2 April. Between 22 and 26 April all dead Plantains, and as many dead *Euphrasia* as possible, were replaced. For this purpose the pots were lifted out of the ground. The pots have gaps at the bottom and rot after plunging, so that the roots of the plants can develop more or less freely. The mortality varied somewhat from one sample to another, and of the 448 plants present after replacements had been made
on 26 April 337 were alive on 19 July. Pronounced signs of establishment on the host in a few plants, and slight symptoms in many others, were seen on 12 May, 7 to 8 weeks after potting up. About half of the Euphrasias present were established or probably established by 18 May, including many which were planted as replacements on 22 to 26 April, but including none in the few pots in which both Euphrasia and host were replaced. By 19 July only two plants out of 337 were not established. Nearly all the plants that were healthy became extraordinarily large-leaved and vigorous. Generally they were much more luxuriant than the most vigorous plants to be found in nature. A view of the plants is shown in Fig. 52, p.132.

The host-plants were also very vigorous and were several times cut back severely to stop them from covering the Euphrasias and to check their growth somewhat. The last time the severe cutting-back was done, some Euphrasias were getting bushy, and the Plantains overshadowed by them did not recover their vigour. Subsequently these Euphrasias appeared to suffer somewhat. It appears that, when the Plantain is liable to be overshadowed, some of its leaves that project out beyond the shadow of the Euphrasia should be left untrimmed.

Many of the Euphrasias that died did so after establishment on the host. Death was preceded by prolonged wilting; the first wilting plant was seen on 25 June. In July many plants showing signs of wilting were tied up to small
FIG. 52: Cultivation of *Euphrasia* (mostly *E. nemorosa*) in the garden in 1953 with *Plantago lanceolata* as host-plant.
sticks. Most of the wilting plants were very heavy, and the stems were decumbent at the base and did not carry the weight of the plants. It was thought that the wilting was caused by the fraying of the base of the stem, which resulted from the twisting of the stem as the head of the plant blew about in the wind. The movement of the plants in the wind doubtless brought on the wilting symptoms, but the stems may previously have been weakened by attacks of damping-off fungus at the seedling stage, as weaknesses at the bases of the stems were found in _E. nemorosa_ cultivated in the greenhouse in 1955. The weak zone in these plants was discoloured and thinner than normal, and it sometimes broke when a plant was pulled up for pressing. In the absence of wind, the weakness had no effect on the vigour of the plants, some of the largest having the weakest stems. Damping-off had been particularly troublesome among the young seedlings of this population.

Other Euphrasias were also tried with other hosts in 1953. The treatment was slightly different, as follows:

1. two Euphrasias were planted in each "whalehide" pot;
2. the hosts were pieces of divided plants with some roots present; one piece per pot was used;
3. after potting up (25 March, 31 March and 2 April) the plants were left in a sheltered enclosure in the garden and not plunged in the experimental bed until 22 April; on this date some, but not all, of the dead Euphrasias were replaced. The Euphrasias were _E. vigursii_ (El97), of which there were 20 pots with
Koeleria gracilis as host; Euphrasia sp. (cf. E. micrantha, E231), of which there were 10 pots with Koeleria gracilis; and two samples of E. anglica (E168 and E219), of each of which there were 10 pots with Luzula campestris.

The most successful populations were the two of E. anglica. On 31 May (about eight to nine weeks after potting up) 12 out of 16 survivors of one of these samples (E219) and 6 out of 16 of the other (E168) showed signs of establishment on the host. On 21 June the corresponding figures were 16 out of 16 and 13 out of 15. The plants grew vigorously, having fairly large leaves and numerous branches. They did not attain anything like the size of the largest plants of E. nemorosa grown outdoors with Plantago lanceolata (pp.130-131), but were better developed than any seen in their parent wild populations. Some were about comparable with one of the samples of E. nemorosa with P. lanceolata (E151A) which shared with E. anglica a relatively low node of flowering that probably prevented the exuberant growth found in late-flowering forms of E. nemorosa.

On Koeleria gracilis, E. cf. micrantha (E231) made little progress. On 26 June 2 plants out of 16 showed signs of being established on the host, though 5 others (apparently not established) were flowering. On 31 August 3 out of the 12 survivors may have been established. None of the plants, however, was as vigorous as the more vigorous plants of the same sample grown with Sagina in the green-
house (Fig. 35, p.90). In *E. vigursii* the first signs of establishment (in one plant) were seen on 18 May; on 26 June, 16 out of 33 plants may have been established, and on 30 July 25 out of 28. They were, therefore, about five weeks behind *E. anglica* in becoming established. They eventually became about as vigorous as the plants of their parent wild population, and they varied quite considerably in vigour.

The "whalehide pot" method of cultivation was again used at Cambridge in 1954. In one experiment, populations of *E. nemorosa* and *E. ?confusa* were grown, but mortality was very heavy. The survivors again grew very vigorously on *Plantago lanceolata*. In another experiment, using seed of *E. nemorosa* (E151A) from the main 1953 garden experiment, the Euphrasias grew with a higher percentage of success on *P. lanceolata*; many plants were hybrids between E151A and other forms of *E. nemorosa* used in the 1953 experiment. In addition, *E. borealis* (E329) was grown with four different hosts, with the results described on p.100 and in Table 9, p.99.

In 1956 the method was again used to see whether more success could be obtained than previously at Cambridge. *E. nemorosa* (E608) was grown in four rows of 10 pots each. The 40 pots were each planted with one Euphrasia and one *Plantago lanceolata* seedling on 4 April. One row of 10 pots selected at random was planted out on 7 April, and the other three rows were planted later. Until 10 May dead
plants were replaced both before and after they were planted in the ground.

Table 17 shows that more Euphrasias died in the first week after planting-out, if planting-out was early, than if it was late. A number of seedlings also died in the period before planting-out; during this time the pots were kept in a cool greenhouse, but those for row C were transferred to a frame on 20 April. As the mortality of the Euphrasias was nearly as severe in the greenhouse and frame as it was outdoors, little advantage, it will be seen, was gained by late planting-out.

**TABLE 17: PROGRESS OF OUTDOOR CULTIVATION OF E. NEMOROSA IN 1956**

<table>
<thead>
<tr>
<th>Euphrasias</th>
<th>Row</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date planted out</td>
<td></td>
<td>7 April</td>
<td>20 April</td>
<td>10 May</td>
<td>10 May</td>
</tr>
<tr>
<td>No. dying in week after planting out</td>
<td></td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total no. dead by 17 May</td>
<td></td>
<td>16</td>
<td>13</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>No. alive on 4-5 June</td>
<td></td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>No. established on host by 4-5 June</td>
<td></td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>No. alive on 26 June</td>
<td></td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>No. established on host by 26 June</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>No. alive on 11 August</td>
<td></td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

+ Counted from 4 April.
This experiment also included rows of 10 pots of some other species with *P. lanceolata*. The pots were prepared on 4 April and planted out on 10 May. A few Euphrasias became established on the host, and some of them were quite vigorous. The species were as follows:

- **E. confusa** (E601): 5 became established (10 replaced after 10 May).
- **E. occidentalis** (E571): 4 or 5 became established (8 replaced after 10 May).
- **E. pseudokernerii** (E606): 5 became established (6 or 7 replaced after 10 May).
- **E. micrantha** (E604): all died in week after planting out and were not replaced.

This method of cultivation was unsuccessful in Cambridge because of the high mortality of the Euphrasias. This may have been due to the long periods of sunny weather accompanied by cold dry winds that usually occur there in April. In 1956 there was a drought in May too.

A plant of *E. nemorosa* from this experiment weighed approximately 21 gm. after pressing. The plant was about as large as any grown in the garden, and therefore similar in size to many of the plants of *E. nemorosa* grown outdoors in open ground in 1953. These, as has been stated (p.131), were far more vigorous than the most luxuriant plants of *E. nemorosa* that can be found in nature. Wild plants are only about as vigorous as the plants of *E. nemorosa* grown in pots with various hosts and weighing up to 1.6gm. (Fig. 50, p.124).
Summary of Observations on Cultivation.

In 1952 the number of leaves and leaf teeth and the number and position of flower buds, flowers and branches were recorded for *E. pseudokernerii* grown from seed in pots in the greenhouse. With no host the plants were dwarf, but they flowered; some plants were similarly dwarf when hosts were provided, but others were much more vigorous and were presumably established on the host. Dwarf and vigorous examples sometimes occurred with the same host-plant, and it is therefore unwise to conclude that negative results always indicate unsuitable hosts. However, *Prunella vulgaris* and *Koeleria gracilis* are probably unsuitable as hosts for *E. pseudokernerii*. Some starved-looking *Euphrasia* had haustoria. Good hosts in this trial were *Festuca ovina*, *Plantago lanceolata* and *Pelargonium x hortorum*. The time required for the production of visible symptoms of establishment on the host was 71-95 days, much longer than that recorded by other workers, and longer than in most of my subsequent cultures. Precocious flowering (at the third and fourth nodes in mid-April) occurred in this *Euphrasia* species in 1952 in dwarf plants with and without hosts; only the more advanced seedlings were affected, while the backward ones behaved normally and were finally the largest. This precocious flowering was attributed to very high temperatures in the greenhouse in March and April. Temperature fluctuations were probably also the cause of the resumption of branching after flowering had started in
some plants.

Plants collected as seedlings in April 1952 from the same population of *E. pseudokerneri* grew well on other monocotyledonous and dicotyledonous herbs with which they were potted up. Some failures to become established under these circumstances are reported for *E. occidentalis* (E73) and *E. anglica* (E67). Various *Euphrasia* species, collected from April to September 1952, were cultivated successfully in the turf in which they had been growing naturally. In these turves, leguminous hosts appeared to be responsible for very vigorous growth of some *Euphrasia* plants which showed signs of having a rich nitrogen supply.

In 1953 *E. pseudokerneri* and *E. anglica* were grown with 21 host species in pots outdoors. Many plants appeared to become established; visual estimations of vigour were used to compare them. For *E. pseudokerneri* dicotyledons (especially Leguminosae) predominated among the best hosts, and for *E. anglica* there was a suggestion of a preponderance of monocotyledons among the best hosts. Plants of both species flowered and fruited with no host. Some disagreements between the performance of *E. pseudokerneri* in this experiment and in 1952 confirm the inconclusiveness of negative records. As in 1952, haustoria were sometimes found where Euphrasias were poorly developed.

In 1954 *E. occidentalis*, *E. nemorosa* and *E. borealis* were compared on three hosts in the greenhouse, and *E. borealis* was also tried outdoors on the same three hosts
and one other. The growth of the Euphrasias was compared by measuring the total length of stem and branch produced by each plant. Definite indications were obtained that *Trifolium dubium* is a poor host for all three species indoors, and for *E. borealis* outdoors. *Briza media*, the host which was tried outdoors only, was a failure. With *E. borealis*, *Medicago lupulina* was better indoors than outdoors, while the reverse was true of *Plantago lanceolata*. Outdoors the better hosts (from the point of view of the vigour of the parasite) gave better survival, more establishment and more flowering by a given date. The time required for the appearance of establishment symptoms, indoors and outdoors, was 26-62 days.

Some of the *Euphrasia* species already mentioned, as well as many others, appeared to become established on various hosts in pots in 1953 and 1954 (see list on pp.102-104). Leguminosae are shown to be good hosts, and *E. micrantha* (rarely seen away from *Calluna vulgaris* or *Erica cinerea* in nature) grew well on *Medicago lupulina*. *Carex carvophyllea, Koeleria gracilis* and *Leontodon autumnalis* are three hosts which were tried extensively and were definitely unsatisfactory. The species cultivated in 1953 and 1954, with the exception of *E. rivularis*, retained the characters of their parent wild populations. When the number of Euphrasias per pot varied, it was found that the size of the plants was smaller the greater the number of plants per pot.
Quite good growth was indicated for some species with no host (pp.109-112). The actual behaviour (branching, flowering, fruiting, etc.) of many host-less plants is described in detail; flowering and fruiting are recorded in several cases. The largest plants occurred where the number per pot was small.

In a culture in 1953 five species of *Euphrasia* were grown on *Sagina apetala* and one on *S. procumbens*. Four plants of each species were grown in each pot of 3½ inches diameter. Of the 24 plants, two failed to show signs of establishment; the rest grew quite well.

In the biggest host trial, carried out in 1955, *E. nemorosa* was tried with four host species and with no host. The minimum time for establishment on the host, judged visually, was 29 days. Establishment was more rapid and occurred in a greater percentage of plants as the distance between the *Euphrasia* and host-plant decreased. When hosts were present, the number of Euphrasias per pot made no difference to the average weight of the Euphrasias after pressing. Comparisons between hosts are summarized in Table 16, p.127. There was no difference between speed of establishment on the different hosts, but Table 16 shows that there were differences in the percentage becoming established, average weight, variation in weight, and mortality.

With no host, though mortality was very low, the performance of the Euphrasias was in other ways inferior to
that on three of the four hosts used. With no host the average weight of Euphrasias was less with six plants per pot than with one to four per pot. Some plants with no host exceeded 17 cm. in height, and most flowered and fruited, including plants alone in their pots. Single plants grew no worse in 3½-inch diameter pots than in larger pots which contained 2½ times as much soil.

Mortality of unestablished Euphrasias in the presence of a host is high, suggesting that the competition offered by the host-plant has an adverse effect on their survival.

For the cultivation of *E. nemorosa*, the experiment showed that *Medicago lupulina* was superior as a host to *Hieracium pilosella*, *Plantago lanceolata* and *Bromus erectus*. *M. lupulina* produced symptoms of a good nitrogen supply in the Euphrasias.

Two methods of outdoor cultivation were tried. In the first, wild seedlings were collected and planted out between plants of *Plantago lanceolata* planted in rows. All the seedlings of *E. occidentalis* died, but a few of *E. pseudokernerii* lasted until September, and two appeared to become established on the host. In the second method *Euphrasia* seedlings and plants or seedlings of the host were planted in bituminized paper pots, which were then planted in the ground. Several *Euphrasia* species were grown on *Plantago lanceolata*, and *E. nemorosa* grew extraordinarily vigorously on it. *E. anglica* grew well on *Luzula campestris*, and *E. vigursii* grew fairly well on
Koeleria gracilis, but E. cf. micrantha showed only slight signs of establishment on this host. This second method was quite successful at Leicester, but it has not been of much use at Cambridge owing to heavier mortality of the Euphrasia seedlings. Cutting back the host-plants is particularly necessary outdoors, where they grow more vigorously than in pots.

My work bears out the results of previous workers, except that I frequently and easily obtained flowering and fruiting of Euphrasias with no host, and also that the occurrence of precocious flowering has not been reported before. Extensive use was made of indoor and outdoor cultivation methods involving transplantation of seedlings germinated in cultivation or in nature; this seems to have been scarcely attempted before. It considerably facilitates the use of Euphrasia as experimental material. That the host range is wide, and includes weed species which the Euphrasias would not meet with in nature, had been demonstrated before, but the present work extends the host range, and demonstrates growth on Pelargonium, another plant with which Euphrasia could not come into contact naturally. There are pronounced differences in the behaviour of Euphrasias with various hosts. The special virtues of certain Leguminosae are reported, but one of the few plants which definitely seems to be useless as a host is Trifolium dubium.
BREEDING STUDIES ON EUPHRASIA

I. Chromosome Numbers of Euphrasia Species.

Most of my work on this subject has been published (Yeo, 1954) in a paper which described chromosome counts of fifteen British forms of Euphrasia, comprising thirteen species. The counts were made at the stage of pollen mother cell meiosis. Staining was by the acetocarmine squash method, and preparations were made permanent with "Euparal", after irrigation with triacetin.

The numbers \( n = 22 \) and \( n = 11 \) were observed. All previously reported numbers were listed, and all known numbers were shown, in a table on p.105 of the paper. In addition to the regular numbers, \( E. \) brevipila var. notata showed pairing failure in one pair of chromosomes, and plants were found with \( 2n = 44 + 1 \), presumably caused by this pairing failure; also a single triploid individual was observed, which most probably forms 11 bivalents and 11 univalents at metaphase. This plant was a hybrid between \( E. \) micrantha and \( E. \) anglica.

The relation of chromosome numbers to classification was discussed. The division between diploids and tetraploids coincides with one of the divisions of Pugsley's classification, the Series Hirtellae being diploid and other groups tetraploid. The raising of the Hirtellae to the rank of Subsection was recommended on morphological as well as cytological grounds. The anomalous forms \( E. \).
brevipila var. notata and var. reavensis are tetraploids; this confirms their exclusion by Pugsley from the Series Hirtellae.

The existence of diploid and tetraploid series makes it possible for certain pairs of species to grow in company and remain distinct. This they frequently do; triploids, however, are very rare.

It was concluded that differences of chromosome number are not the cause of the multiplicity of Euphrasia species, but that a chromosome survey of other groups of Euphrasia, particularly those within the Section Semicalcaratae, is needed.

The paper included a few photographs of chromosomes; others illustrating the reported chromosome counts are given here in Figs. 53-57, pp.146-150. One stage not previously illustrated is late Anaphase I in a tetraploid (Fig. 53f).

Some cytological work has also been reported on some forms of Euphrasia considered to be of hybrid origin (Yeo, 1956).

Fig. 58, p.151, illustrates some hitherto unpublished chromosome counts. E. borealis (E247) has been found to be a tetraploid, with about 22 bivalents at Metaphase I in pollen mother cell meiosis, though no cells were found which could be completely interpreted with certainty. As well as cells that were probably normal there were some that showed one univalent, and some that showed two, at Metaphase I.
FIG. 53: Pollen mother cell meiosis in *Euphrasia*. x 1000 or x 800.
a. *E. scotica* (E261)  
b. *E. foulaensis* (E227)  
c. *E. marshallii* (E311)  
d. *E. curta var. rupestris* (E100)  
e. *E. occidentalis* (E192)  
f. *E. occidentalis* (E192)  
a. to e. Early Anaphase I (AI), 22 bivalents in each.  
f. Late AI, 22 + 22 chromosomes.
FIG. 54: Pollen mother cell meiosis in *Euphrasia*. x 1000 or x 800.

- a. *E. nemorosa* (E134)  
- b. *E. nemorosa* (E149)  
- c. *E. pseudokerneri* (E143)  
- d. *E. confusa* (E175)  
- e. *E. brevipila* (E245)  
- f. *E. brevipila* (E245)

All show early AI. a. to e. show 22 bivalents each. f. 20 bivalents, probably 4 univalents.
FIG. 55: Pollen mother cell meiosis in Euphrasia brevipila var. notata. x 1000. All early AI.

a. E254, 22 bivalents
b. E254, 2 univalents, bivalents unclear

c. E262A, 22 bivalents
d. E254, 22 bivalents & 1 univalent

E. anglica (E100), analagous
E. anglica (E100), All, 11 + 11 chromosomes

E. anglica (E150), early AI
E. anglica (E190), All, 11 + 11 + (probably) 22 chromosomes
FIG. 56: Pollen mother cell meiosis in *Euphrasia*. x 1000 or x 800.

a. *E. brevipila* var. *reyensis* (*E304*), early AI, 22 bivalents
b. *E. anglica* (*E168*), early AI, 11 bivalents

c. *E. anglica* (*E168*), Metaphase II (MII), 11 + 11 chromosomes
d. *E. anglica* (*E168*), AII, 22 + 22 chromosomes

e. *E. anglica* (*E150*), early AI
f. *E. anglica* (*E150*), AII, 11 + 11 + (probably) 22 chromosomes
FIG. 57: Pollen mother cell meiosis in Euphrasia. x 1000 or x 800. Four micrographs on right-hand side.

- E. anglica (E157), E. rivularis (E123), AII, early AI, 11 bivalents
- E. anglica x E. micrantha (E185C), probably 11 bivalents and 11 univalents, 2 lagging univalents
- E. anglica x E. micrantha (E185C), Telophase I, with 11 + 11 + 22 chromosomes

MI (see Yeo, 1954, Fig. 1, for interpretative diagram)
FIG. 58: Pollen mother cell meiosis in *Euphrasia*. x 800.

a. *E. borealis* (E247), MII, showing two half-chromosomes (chromatids) on the left and one on the right (a second is present on the right but is not distinguishable in this photograph).

b. *E. salisburgensis var. hibernica* (E230), MII, 22 chromosomes on right-hand side.

c. *E. occidentalis*, long-glandular form (E342), MI, 22 bivalents.
E. borealis is illustrated in Fig. 58a, which shows half-chromosomes at Metaphase II; these presumably result from the division of univalents at first division.

E. salisburgensis var. hibernica (E230) is shown to be a tetraploid (Fig. 58b).

Fig. 58c is taken from a form which has a long-glandular indumentum, and which proved to be tetraploid. It was collected on waste ground in Perranporth, Cornwall, and looked like a rather tall form of E. occidentalis, which is normally compact and short-glandular. E. occidentalis was found on nearby dunes, and was normal in habit, but in some areas many plants were long-glandular. This may be the result of hybridization with E. curta, a species with a dense, long and eglandular indumentum, which I did not find at Perranporth. That these long-glandular forms of E. occidentalis have an indumentum like that of the diploid species of Euphrasia (Series Hirtellae) is doubtless only a coincidence. Other examples of tetraploid long-glandular forms are E. brevipila vars. notata and reavensis.

A list of all the chromosome numbers now known in Euphrasia is given in Table 18, p.153. All the counts are my own unless otherwise indicated.
# Table 18: Known Chromosome Numbers of Euphrasia; Species Arranged in Systematic Order According to Pugsley (1936)

## Section Semicalcaratae

### Subsection Angustifoliae

<table>
<thead>
<tr>
<th>Species</th>
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<tbody>
<tr>
<td>salisburgensis</td>
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</tr>
<tr>
<td>salisburgensis var. hibernica</td>
<td>22</td>
</tr>
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### Subsection Ciliatae

#### Series Pectinatae

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#### Series Nemorosae

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<tr>
<td>confusa</td>
<td>22</td>
</tr>
<tr>
<td>occidentalis</td>
<td>22</td>
</tr>
<tr>
<td>pseudokernerii</td>
<td>22</td>
</tr>
<tr>
<td>curta var. rupestris</td>
<td>22</td>
</tr>
<tr>
<td>marshallii</td>
<td>22</td>
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#### Series Latifoliae

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<tr>
<td>frigida</td>
<td>22</td>
</tr>
<tr>
<td>foulaensis</td>
<td>22</td>
</tr>
<tr>
<td>scotica</td>
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</tr>
<tr>
<td>minima</td>
<td>22</td>
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#### Series Brevipilae

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<td>brevipila</td>
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</tr>
<tr>
<td>brevipila var. notata</td>
<td>22</td>
</tr>
<tr>
<td>brevipila var. reayensis</td>
<td>22</td>
</tr>
<tr>
<td>borealis</td>
<td>22</td>
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#### Series Hirtellae

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</tr>
<tr>
<td>anglica</td>
<td>11</td>
</tr>
<tr>
<td>rivularis</td>
<td>11</td>
</tr>
<tr>
<td>rostkoviana</td>
<td>11</td>
</tr>
<tr>
<td>montana</td>
<td>11</td>
</tr>
</tbody>
</table>

1. von Witsch (1932).
3. Löve (1948).
II. The Flower and Pollination

Previous Work on Pollination.

Wettstein (1896) described three types of Euphrasia flower in connection with pollination. In the species with large flowers attaining 12-15mm. 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 in which they transmit pollen to other flowers by means of insect visitors. The morphology of the flowers is illustrated in Fig. 59. Nos. 13 and 14 of this illustra-

FIG. 59: Flowers of Euphrasia, reproduced from part of Plate 2 of Wettstein's Monograph of the Genus Euphrasia (1896).
tion show a flower of *G. rostkoviana* when it first opens. The nectary is situated at the base of the ovary on the lower side. 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. This stage is shown in nos. 15, 16 and 17. 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. mulchella*, in which the corolla reaches a length of 8-10mm. 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 (Fig. 59, nos. 18 & 19). 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 (no. 20). 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 observed that the pollination methods of these types of flower corresponded with their size. He also considered that the dependence of the large-flowered species on insect-pollination was related to their occurrence in regions rich with insects, there being no large-flowered species in the arctic regions.

According to Pugsley (1930), Chabert considers that the corolla tube lengthens in all Euphrasias, though not very appreciably in Wettstein's Parviflorae. Pugsley's own observations (1930) also tended to confirm Wettstein's. He says, "In all Eyebrights the whole corolla apparently continues to grow, after first opening, until the dehiscence of the anthers, this growth involving some elongation of the corolla tube. In the Parviflorae the growth of the tube is generally proportionate to that of the lips, and when it ceases, the tube does not appreciably exceed the calyx-teeth. There are probably some modifications of this rule, and I believe that a relatively greater elongation of the
tube commonly takes place in very small flowered plants like *E. minima*. In Wettstein's Grandiflorae the elongation of the tube is greater than that of the remainder of the corolla, so that at maturity the tube obviously exceeds the calyx teeth and the corolla becomes distinctly exserted."

Pugsley was inclined to think that even among the Grandiflorae autogamy frequently takes place. He considered that the expansion of the corolla leads to the bringing of the anthers into contact with the stigma, that the more curved the style the sooner contact is made, and that if the stigma is still receptive self-pollination then takes place.

Müller (1883) describes the flower fully, and refers to the dark violet lines and orange spots on the corolla as pathfinders. He states that honey is found at the base of the tube; that the pollen is smooth and powdery as in *Odontites serotina* (*O. verna* ssp. *serotina*), and falls on to the head or proboscis of the visitor; that if the spines on the anthers are touched all the anthers are shaken, causing the pollen to fall out; and that the hairs on the anthers prevent the scattering of pollen at the sides and ensure its falling on the head of the insect. He describes a large-flowered form, probably *E. montana*. The stigma protrudes before the anthers are ripe, and even after they are ripe it is the first part to be touched by insect-visitors. He says that in the absence of insects the flower is incapable of self-fertilization. In a small-flowered
form, probably *E. micrantha*, the stigma is behind and above the anthers and escapes being touched by insects. Then the style elongates and brings the stigma to where it can be touched by insects or where pollen can fall on it from the anthers. He surmised that intermediate forms of flower exist.

Knuth (1909) classified the flowers of *Euphrasia* as "Hymenopterid, with concealed nectar". He stated that A. Schulz classified the flowers of *Euphrasia* into seven forms. It is not necessary to repeat these here; the first and last correspond more or less with Müller's two types. Two points of interest may be noted; Schulz said that in large-flowered forms the stigma protrudes from the bud, that large-flowered forms have a well-developed nectary, and that in small-flowered forms the nectary is less well-developed or absent. Knuth quotes Kerner to the effect that automatic self-pollination can eventually take place in the large-flowered *E. rostkoviana*, *E. tricuspidata* and *E. versicolor*. In *E. minima* Schulz found the mature stigma beneath the anthers before the flower opened. Kerner observed the stigma bending down after the flower opened, so as to be in the line of fall of the pollen.

A list of insect visitors to the flowers of *Euphrasia* is given in Table 19, p.159. The records given by Knuth (1909) were accompanied by some observations on the behaviour of the insects. Thus the following were sucking nectar: *Bombus lapidarius*, *B. muscorum*, *B. agrorum*, *B. pratorum*,
### TABLE 19: INSECT VISITORS TO THE FLOWERS OF EUFRASIA.
Records given by Knuth (1909), Müller (1883) and Wettstein (1896). Where identification of Euphrasia is not precise, the locality is given.

<table>
<thead>
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<th>Insect</th>
<th>Euphrasia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diptera.</strong></td>
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</tr>
<tr>
<td>Bombylidae</td>
<td></td>
</tr>
<tr>
<td>Systoechus sulphureus Mk.</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td><strong>Syrphidae.</strong></td>
<td></td>
</tr>
<tr>
<td>Melithreptus taeniatus Mg.</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>Syrphus sp.</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>S. ribesii L.</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>Melanostoma barbifrons Fall.</td>
<td>rostkoviana</td>
</tr>
<tr>
<td>Helophilus pendulus L.</td>
<td>officinalis (N. Frisian Is.)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>large- &amp; small-flowered (Pyrenees)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>officinalis (Scotland)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>salisburgensis</td>
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<tr>
<td><strong>Muscidae.</strong></td>
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</tr>
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</tr>
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<td>officinalis (Alps)</td>
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<td>Unidentified</td>
<td>minima</td>
</tr>
<tr>
<td>Unidentified</td>
<td>large- &amp; small-flowered (Pyrenees)</td>
</tr>
<tr>
<td><strong>Hymenoptera: Apidae.</strong></td>
<td></td>
</tr>
<tr>
<td>Apis mellifera L., worker</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>Bombus agrorum (Fab.), worker</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>B. hortorum (L.)</td>
<td>officinalis (E. Frisian Is.)</td>
</tr>
<tr>
<td>B. lapidarius (L.), worker</td>
<td>officinalis (E. Frisian Is.)</td>
</tr>
<tr>
<td>B. lapidarius (L.)</td>
<td>officinalis (Bremen)</td>
</tr>
<tr>
<td>B. muscorum (L.), male, female and worker</td>
<td>officinalis (E. Frisian Is.)</td>
</tr>
<tr>
<td>B. muscorum (L.)</td>
<td>officinalis (Bremen)</td>
</tr>
<tr>
<td>B. pratorum (L.), worker</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>B. soroensis (Fab.)</td>
<td>officinalis (Alps)</td>
</tr>
<tr>
<td>B. soroensis var. proteus Gerst.</td>
<td>officinalis (N. Frisian Is.)</td>
</tr>
<tr>
<td>Bombus (&quot;two Humble-bees&quot;)</td>
<td>officinalis (Scotland)</td>
</tr>
<tr>
<td>Colletes impunctatus Nyl.</td>
<td>officinalis (E. Frisian Is.)</td>
</tr>
<tr>
<td>C. marginatus L.</td>
<td>officinalis (E. Frisian Is.)</td>
</tr>
<tr>
<td>Epeolus variegatus (L.)</td>
<td>officinalis (E. Frisian Is.)</td>
</tr>
<tr>
<td>Halictoides paradoxus Mor., female</td>
<td>large-flowered (Austria)</td>
</tr>
<tr>
<td>Halictus minutissimus K.</td>
<td>officinalis (Alps)</td>
</tr>
<tr>
<td>Nomada lateralis Panz., female</td>
<td>officinalis (Westphalia)</td>
</tr>
<tr>
<td>Psithyrus rupestris (Fab.), female</td>
<td>officinalis (Austria)</td>
</tr>
</tbody>
</table>
Apis mellifera, Nomada lateralis, Colletes impunctatus, G. marginatus, Systoechus sulphureus, Melithreptus taeniatus, Syrphus sp., and the very small Halictus minutissimus which was creeping right into the flowers. One of these bees, G. marginatus, was collecting pollen as well as nectar.

Description of the Flower.

The morphology of the Euphrasia flower is well shown by the illustrations in Wettstein's monograph (1896), reproduced in Fig. 59, p. 154. Wettstein also describes the flower fully, and my own observations agree well with his. A few facts not observable in the illustrations may be mentioned. The ground colour of the corolla may be white or, rarely, straw-yellow; often it is some shade of violet, lilac-pink or red-purple. This colour may be evenly distributed, but usually it is stronger in the upper lip than the lower, and is sometimes especially strong in the lobes of the upper lip; in addition, it nearly always becomes more intense with the increasing age of the flower. If the lilac colour is weak, the flower may be white (or yellow) initially, and become lilac (or greyish lilac). The veins are usually dark violet, but the extent to which this is so varies. Occasionally the colour appears to have "run" from the veins and flooded the area nearest them with deep purple. The coloured veins are usually simple, but occasionally feathered. A circular spot near the base of
the lower lip and a mark on the lower side of the tube just inside the throat range in colour from faint yellow to bright orange; these marks are occasionally absent. When flowers are dried the lilac or purple coloration tends to become greyish violet (i.e. bluer, as well as greyer) or, in very intensely coloured flowers, blue-black. Blue does not occur in living flowers. The anthers are dark red and turn brown as they dehisce. In a gathering of *E. curta* from Anglesey, made by Mr. J. E. Lousley, anthocyanin was quite lacking from all parts of the plants, and the anthers were light brown. Regarding the enlargement of the corolla, my own impressions can well be summed up in the words of Pugsley that I have quoted (p.156), except that I have found the enlargement of the corolla to be more prolonged than he said.

The illustrations in Fig. 59, p.154, show that the filaments are inserted near the throat of the corolla, and that their curvature holds the anthers in a vertical position. The four anthers are held together by the flexuous hairs on them, according to Wettstein (1896), who says they are held together "at least at the beginning of anthesis". In each anther-loculus dehiscence begins at the proximal, free end, but it does not run the full length of the loculus. After dehiscence a quantity of pollen falls onto the lower side of the throat, and some remains among the flexuous hairs that border the orifices. Further conclusions about the flower can be drawn from the detailed
observations that follow, which were made on plants in cultivation.

E. pseudokerneri from Holywell Mound, Lincs. (E42 and E70).

Table 20, p.163, gives measurements made of the growth of flowers in 1952. The length of the flowers was measured from the base of the calyx to the tip of the upper lip, and the node of the flower was counted from the first floral node upwards. These figures give an impression of an increase in the size of the whole flower continuing through most of its life. The two decreases recorded may represent error in measuring, or a real shrinkage of the flower shortly before it was shed. My experience in collecting flowers for mounting suggests that such shrinkage quite frequently occurs.

The flowers at the second floral node of plant 1 (Table 20) were just opening about 10 a.m. on 9 May 1952; their lower lips were still up-curved, but there were gaps between them and the upper lips.

By 12.15 the lips had uncurled and the first set of measurements was taken. The styles protruded from the upper lip by over 1mm. and were bent so that the distal portion was at 90° to the upper lip and about parallel to the lower. There was some pollen on the lower lip of flower A. At this time the flowers at the first floral node were larger, being 10mm. long; the lower lips measured 7mm. and 6.5mm. in length.
### TABLE 20: ENLARGEMENT OF THE COROLLA OF E. PSEUDOKERNERI (E70)

(Measurements in mm.)

<table>
<thead>
<tr>
<th>Node of Plant Flower</th>
<th>Date</th>
<th>9/5</th>
<th>10/5</th>
<th>11/5</th>
<th>12/5</th>
<th>13/5</th>
<th>14/5</th>
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<tbody>
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<td>Time</td>
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<td>12.40</td>
<td>11.20</td>
<td>16.15</td>
<td>17.15</td>
<td>13.00</td>
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<td>8.5</td>
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<td>9.5</td>
<td>9.5</td>
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<td>6.0</td>
<td>6.0</td>
<td>6.5</td>
<td>6.5</td>
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<td>Width of Lower Lip</td>
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<td>8.0</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
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<td>Length of Flower</td>
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<td>9.5</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>Length of Lower Lip</td>
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<td>5.0</td>
<td>5.0</td>
<td>6.0</td>
<td>6.0</td>
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</tr>
<tr>
<td></td>
<td>Width of Lower Lip</td>
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<td>8.0</td>
<td>8.0</td>
<td>8.5</td>
<td>8.5</td>
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</tr>
<tr>
<td>2</td>
<td>Length of Flower</td>
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</tr>
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<td></td>
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<td>5.5</td>
<td>6.0</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Length of Upper Lip</td>
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<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Width of Lower Lip</td>
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<td>16.00</td>
<td>11.45</td>
<td>17.45</td>
</tr>
<tr>
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<td>Length of Flower</td>
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<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
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<td>Width of Lower Lip</td>
<td>-</td>
<td>6.5</td>
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</tbody>
</table>

<table>
<thead>
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<td>19.15</td>
<td>21.00</td>
<td>20.45</td>
</tr>
<tr>
<td>2</td>
<td>Length of Flower</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>Length of Lower Lip</td>
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<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Length of Upper Lip</td>
<td>3.0</td>
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<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Width of Lower Lip</td>
<td>6.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>
At 16.45 the measurements of the flowers at the second floral node were unchanged, except that the lower lip of flower B was about 4.5 mm. long.

At 9.30 on the second day (10 May) the flowers at the second floral node were laterally compressed and the lower lips folded. The same partial closure was seen on that day in a flower which had also opened on 9 May on another plant. It was not shown on 10 May, however, by the flowers at the first floral node of plant 1, where the lower lips were nearly flat. It appears, therefore, that the young flowers partially close at night, but that the older ones do not.

The flowers at the second floral node had opened out by 11.00 on the second day, there had been no change in the styles, and flower B had still shed no pollen.

On the third day (11 May) at 11.20 these flowers were fully open, the styles being still down-curved and now about 1.5 mm. in front of the anthers. The anthers of both flowers were seen to be dehisced, and there was pollen in the tube of flower B.

By 17.15 on 13 May, two days later, the stigmas were a little nearer the anthers, the styles being slightly more curved. (This implies that there was no elongation of the corolla in relation to the style). The stigmas were in the same position the following day.

There was little change on 15 May, and the flowers had been shed by the next morning. The stigmas then appeared
to have no pollen on them, and the styles were brown at the base. (The withering of the styles normally begins at the base). In fact, there must have been a little pollen on each stigma, as three seeds were produced by each capsule in June. However, pollination was presumably incomplete; this may have accounted for the long duration of the flowers and the unchanging position of the stigmas, which were probably curved to their maximum extent and as near the anthers as they could be in this rather large flower.

A few days later the flowers at the first floral node of another plant were observed in the same way on their third and fourth days.

At 12.00 on their third day (22 May) they were both 9.5mm. long, and respectively 8.5 and 9.0mm. across the lower lip. The styles were less curved than in the flowers at the second floral node of plant 1, so that they were nearly horizontal (Fig. 60).

At 11.50 on 23 May the styles were still approximately

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**FIG. 60**: Flowers of two plants of *E. pseudokernerii*.  
*Left* - A flower in its third day, on 22 May 1952.  
*Right* - A flower in its fourth day, on 8 July 1952.
straight, while the flowers at the next node were just opening, and had their styles curved, pointing nearly vertically downwards.

Another flower was drawn on its fourth day on 8 July (Fig. 60, p.165). It was a flower at the tenth floral node of plant 2 (Table 20, p.163). This appeared to be behaving like the flowers at the second floral node of plant 1; the corolla tube had elongated, but the style was still bent down.

On the evening of 5 August, another flower on plant 2 was showing a nocturnal closure, and was therefore presumed to be in its first day. The posterior anthers were not dehisced, and the anterior were probably not dehisced and certainly not more than slightly open. There was almost certainly no pollen on the lower lip. The style was curved through slightly more than 90°, and the stigma was well below, but well forward of, the lower edge of the anthers.

At 17.00 on the next day, the style was curved through less than 90°. All the anther loculi were dehisced, and the stigma was fully covered with pollen.

The complete withdrawal of the stigma, as illustrated by Wettstein, was seen in a pair of flowers of this population of \( E. \) pseudokermeri. At 14.30 on the first day (6 August) the lower lip had just completed its unrolling. In both flowers, the anterior loculi of the front anthers had dehisced, and pollen had been shed onto the lower lip or into the throat. There appeared to be a pollen grain on
one of the stigmas; it had perhaps got there as a result of my shaking the plant accidentally. The positions of stigma and front anthers in one flower of this pair are shown in Fig. 61 (Left). In the other flower the stigma was lower down, this flower being less advanced than its partner.

By 12.00 on the next day, the more advanced flower had reached the state shown in Fig. 61 (Right). The stigma was covered with pollen, and had withdrawn above the anthers which now projected slightly; all the anther loculi had dehisced. At this time the other flower at the same node was as shown in Fig. 62 (Left). Its corolla was shorter

FIG. 61: Part of a flower of *E. pseudokerneri*, showing positions of stigma and front edge of anterior anther. **Left** - Flower newly opened, at 14.30 on 6 August 1952. **Right** - Flower at 12.00 on the next day.

FIG. 62: Part of a flower of *E. pseudokerneri*. **Left** - At 12.00 on 7 August 1952. **Right** - At 17.15 on the same day.
than that of its partner; the front anthers were dehisced, but the posterior ones appeared not to be; there was no pollen on the stigma. By 17:15 on the same day, the style appeared to be more curved, so that the inner edge of the stigma was under the edge of the anthers (Fig. 62, Right) and this part of the stigma now had some pollen on it. The posterior anthers still appeared to be undehisced.

On the third day (8 August) the style of the more advanced flower was turning brown; that of the less advanced flower was only slightly protruding, and the terminal part was nearly horizontal. The posterior pair of anther loculi still did not appear to be dehisced.

On another plant, a flower at the fifth floral node began to open on 6 August. At 17:30 the style was bent through about 90°, and the stigma was well in front of the anthers and about level with their lower edge. There was pollen on the stigma, and a few grains on the edge of the lower lip, but little or none on the part of the lower lip beneath the anthers. This state of affairs could have been caused by a visiting insect or by lateral shaking of the stem.

At 12:15 on the second day, the stigma was covered with pollen and was withdrawn to a point on top of the anthers. The back anthers were not dehisced. The same afternoon, at 17:15, the stigma was still further withdrawn, but the back anthers were not yet dehisced.

At 17:00 on the third day, the stigma was not visible
and the posterior anthers had dehisced.

The anthers were removed from an unopened flower of *E. pseudokerneri* on 4 June 1952, and they were found to be dehisced. The flower looked as if it would have opened the following day. In several other flowers, emasculated just before or just as the flower opened, the anthers had not dehisced.

These observations may be summarized by saying that the stigma is at first in the position shown in Figs. 60 (Right) and 61 (Left), and apparently the flower is not then readily self-pollinated. 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 withdrawn above the anthers. In one pair of flowers the styles were straight on the third and fourth days, but the stigmas were not withdrawn. The elongation of the corolla tube must play a part in the withdrawal of the stigma, but the withering of the style from the base might also contribute, as it probably involves contraction.

The behaviour of the flowers of plants of two other populations of *E. pseudokerneri*, one from Surrey (E77) and one from Suffolk (E143), was similar to the behaviour of those just described. It may, however, be mentioned that a young flower from one of these populations (E77) was tapped on 6 August, and this brought pollen on to the stigma.
E. brevinila (E81).

Eight flowers belonging to six plants were measured. The length of the flowers ranged from 5 to 8.5mm., and the breadth of the lower lips from 4.5 to 7mm. A few observations were made on the 11th pair of flowers of plant 1. The 9th flower of this plant was 7.5mm. long and 7mm. across the lower lip when shed. On 6 August 1952, flowers at the 11th node were opening at 12.00. In both flowers the anterior anthers were dehisced, but at least the back loculi of the posterior anthers were undehisced. The styles were bent through 90°, and were long, so that the stigmas were well forward of, and below, the anthers. Both stigmas were free of pollen. One flower was less advanced than the other, in that the lobes of the lower lip were still curved up. Next day, at 11.45, one flower had the stigma covered with pollen and drawn back into the corolla above the anthers. In the other flower, it had partly withdrawn and was above the level of the anthers, but there was no pollen on it.

The anthers were removed from a flower just about to open. Seven loculi had dehisced, and were thought to have done so naturally.

E. confusa (E209).

The flowers of this population were rather large for the species: ten shed flowers of the wild plants were from 6.5 to 8mm. long, and from 6.5 to 8.5mm. across the lower
lip. Fig. 63 shows parts of the flowers at the first and second floral nodes of one plant. The flower at the first floral node (flower 1) was about three days old and 8mm. across the lower lip; the flower at the second floral node (flower 2), drawn at the same time, was in its first day and the lower lip was 6mm. across. The upper lip of flower 1 was abnormal and resembled a single lobe of the lower lip; the anthers were fully exposed to view. In flower 2 the anthers were visible through the upper lip, which was slightly translucent. Fig. 63 (Left) shows a recurved style with the stigma under the anthers. The position of the stigma in the newly-opened flower in Fig. 63 (Right) is similar to that in the examples of other species already described.

The anthers were removed from a newly-opened flower, and were found to be undehisced. In some other cases, the anterior anthers were dehisced in newly-opened flowers.

FIG. 63: **Left** - Flower 1 of a plant of *E. confusa* (E209), about three days old, on 23 May 1953; upper lip abnormal. **Right** - Flower 2 of the same plant at the same time, in its first day.
E. nemorosa form (El96).

The flowers of this population were smaller than those so far described. They were 5.5 to 6.5 mm. long when shed (8 flowers), 6 to 7 mm. long when attached (3 flowers) and 4.5 mm. across the lower lip (11 flowers). The stigma of a second-day flower was seen to be almost in contact with the lower front edge of the anthers, and it was therefore in a position where pollen could easily fall on it.

E. scotica (El20).

The flowers of this species are very small. In the sample under observation, eight shed flowers were from 4.5 to 6 mm. long and from 2.5 to 3.5 mm. across the lower lip. The lower lips never became perfectly flat, the tips of the midlobes being more or less recurved (Fig. 64, Left). A diagram of the upper lip, anthers and style of the same flower, probably in its first day, is shown in Fig. 64 (Right). These drawings were made in the evening. At least the anterior anthers were then dehisced, and there was a good deal of pollen on and around the yellow spot on the lower lip, and also some on the stigma. The other

FIG. 64: Left - View of the upper surface of the lower lip of a flower of E. scotica (El20) probably in its first day, 5 August 1952. Right - Diagram of the upper lip, anthers and style of the same flower at the same time.
flower at the same node was in its first day, and not fully open. There was pollen on the lower lip and the stigma, which was well pollinated, was in much the same position as in the more advanced flower, but possibly slightly closer to the anthers.

At 10.30 on the next day (6 August 1952), there was little change in the more advanced flower: all the anther loculi were dehisced; the style was probably slightly less bent and the stigma not so close to the anthers; there was perhaps more pollen on the stigma. The unbending of the style may have been due to elongation of the corolla, bringing the anthers forward. In the other flower at this node, all the anther loculi appeared to be dehisced. The style was less bent, and apparently shorter, than in the more advanced flower, so that it was in contact with the outside of the anthers. Three other flowers, in their first day, resembled this one. Later the same day, at 16.45, the flower shown in Fig. 64, p.172, was unchanged. Its partner, and two of the first-day flowers, had the stigma withdrawn a little further. In a flower just beginning to open at this time, the stigma was well down in relation to the anthers, but it was unlikely to receive falling pollen, owing to the steep angle at which the flower was held.

E. micrantha (E112A). Four attached flowers were about the size of those of
**E. scotica** (E120), being from 5 to 5.5mm. long and from 3 to 3.5mm. across the lower lip. The emasculation of two flowers, a day or two before they were ready to open, suggested that the dehiscence of the anthers takes place considerably before the flowers open. In the first flower, emasculated on 12 July 1952, the anthers were all dehisced when removed, and there was pollen on the stigma. The second flower was emasculated on 20 July; some anther-loculi appeared to be aborted, but others had dehisced.

**E. micrantha** (E185A).

Eleven flowers of wild plants were from 5.5 to 6mm. long (probably attached) and from 3.5 to 4.5mm. across the lower lip. On 31 July 1953 an unopened flower was emasculated, and one anther was lost; the other three were intact and undehisced, but brown (and so presumably about to dehisce). On the same day I made a note to the effect that the anthers dehisce and pollination takes place before the flower opens, and I believe this was on the basis of other, unsuccessful attempts at emasculation.

Notes were made of two attempted emasculations on 24 August 1955 (This population was maintained in cultivation over several generations). In one emasculated flower, although it was exceptionally young, the anterior anthers had dehisced. The second flower emasculated was still less advanced, and the anthers were successfully removed undehisced. One day about this time, three other flowers
were emasculated and, though they were as young as possible for the purpose, the anthers had dehisced in all of them. However, on 27 August, a larger flower than those just mentioned was emasculated, and the anthers were undehisced. Thus, there is a variation in the time of anther dehiscence in relation to corolla development.

E. anglica (E76) from Box Hill, Surrey.

Of the diploid forms of Euphrasia of which notes were made on flower-development this population had the largest flowers. Seven flowers on their first day were from 6.5 to 8 mm. long, and one or two days before being shed they were from 7.5 to 9 mm. long and from 6 to 8 mm. across the lower lip.

In a flower in its first day on 12 June 1952, the style was bent to make an angle of about 90° with the upper lip, and the stigma was below, but well forward of, the lower edge of the front anthers. In another flower, in its second day, the style appeared long and was greatly curled, so that the stigma was vertically beneath the anthers. In a flower in its third, or possibly second, day the style was similar to that in the flower of this population described first, except that the stigma was not so far in front of the anthers, perhaps because of elongation of the corolla. An apparently slightly more advanced condition was shown by another third-day flower; here the style was bent through about 90°, but only at the extremity, so that
the stigma was in front of, but above, the lower edge of the anthers. What was probably the most advanced state was seen in a flower which was only in its second day; the style appeared shorter than in the flower just described, it was only slightly bent, and the stigma was against the upper edge of the front anthers. It was, therefore, nearly withdrawn.

Some flowers that were emasculated for crossing were not pollinated until some days had elapsed. One flower, emasculated on 7 September 1952 and pollinated on 9 September, had the style strongly curved in on the latter date. Three flowers emasculated on 17 September were not pollinated until 20 September; they were then found to have their styles bent more than usual and the stigmas were in the position below where the anthers should have been. (A similar effect was noticed about this time in *E. pseudo-kernerii*.)

In flowers about to open, undehisced anthers were frequently found, but in some instances the anthers had already dehisced. In one newly-opened flower, only the anterior anthers had dehisced.

Closing movements were seen in the evening in two flowers of this sample, one of which was known to be in its first day. They were also seen in two rather small first-day flowers of another sample of this species from the same locality. In all cases the flowers became laterally compressed.
E. anglica (E71) from the New Forest, Hampshire.

The flowers of this population were smaller than those of the same species just described, being about 6.5 mm. long and 5 mm. across the lower lip when fully out.

One flower was closed for the night at 18.40 on its first day, 21 May 1952. At 12.30 on the next day the style was strongly curved, so that the stigma was under the anthers. At 12.00 on the third day the position of the stigma was unchanged; pollen had fallen on to the yellow spot on the lower lip. On the fourth day the flower was found shed; the style was still curved down.

Two other flowers opened on 26 May. The style of one was bent down through about 90° and was short, not exceeding the stamens. The style of the other was still shorter, and it was less bent because its distal part rested on top of the anthers. The following day both styles were more curved, so that the stigmas were just touching the lower front edges of the anthers but were not underneath them.

E. rivularis (E123).

The flowers of this diploid species were fairly large, being from 5.5 to 8 mm. long and from 5 to 6.5 mm. across the lower lip. In early July, two first-day flowers closed in the evening by dorsi-ventral, instead of lateral, compression, the lower lips rising up to come into contact with the upper lips.
Abnormal Flowers.

In *E. curta* var. *rupestris* (E100), cultivated in 1952, two flowers at successive nodes had capsules exceeding their rather short calyces, but each bore a perfect style and pollen-free stigma, complete with glandular hairs. Each capsule had a translucent or brownish membrane round it, more or less shredded at the top, suggesting that the capsule had burst through an undeveloped corolla, although just possibly the corolla had developed and been eaten by a slug. The two capsules were abnormally large, considering the state of the stigmas. A similar corolla-less flower had been noticed a few days previously in *E. anglica* (E135A). When the style of this flower was rotting at the base, the capsule was not larger than it usually is at the end of flowering. The stigma had lost its glandular hairs, and appeared to have no pollen on it.

In *E. confusa* (E209), cultivated in 1953, one plant had two abnormal flowers at the fifth node and one at an earlier node; the upper lips were deeply divided into two lobes resembling the lobes of the lower lips. At the same time, another plant had an abnormal flower which has already been described (p.171 and Fig. 63, Left). Less extreme states of the first of these abnormalities of the upper lip have been seen occasionally in other plants.

Extra lobes on the lower lip are quite frequent in wild and cultivated plants; there may be one to three extra lobes, and usually they are not all equal in size and do not
all lie in quite the same plane.

Another abnormality was an irregular serration of the edge of the corolla in a plant of *E. pseudokerneri* (E609), grown in 1957, which had somewhat contorted leaves and was evidently diseased.

**Summary of Flower Observations.**

In the foregoing account, diploid species have been separated from tetraploid, but it is clear that flower behaviour follows the same pattern in both groups, being dependent on the size of the flowers.

A summary has already been given of the observations on *E. pseudokerneri* from Holywell Mound (p.169) and it has been mentioned that other populations of *E. pseudokerneri* resembled this one. Basically, the sequence of events in this species seems to be that the corolla opens and the stigma is then low down but clear of the anthers. The latter dehisce some time after the flower opens. Later some or all of the following occur: 1) the style straightens out; 2) the stigma withdraws into the corolla above the anthers; 3) the corolla tube elongates; 4) there is a slight protrusion of the anthers. The style rarely seems to be able to bend enough for the stigma to be pollinated by pollen falling directly from the anthers. The inter-relationships of these changes are not clear. It seems as though lack of pollination may prevent the straightening of the style by internal control. However, the straightening of the style may be assisted by the anthers coming forward
and lifting up the distal part of the style. The withdrawal of the style is probably largely due to the elongation of the corolla, but the withering of the style at the base could also contribute. *E. pseudokerrii*, together with *E. anglica* (E76) and *E. brevripila*, appears to be intermediate in behaviour between the large-flowered and the medium-flowered species described by Wettstein (see pp.154-155), since the stigma very occasionally came down to where pollen could fall directly on to it, and sometimes the anthers had already dehisced when the flower opened. The medium-sized flowers of *E. confusa*, the other *E. anglica* population (E71) and *E. nemorosa* were similar to Wettstein's medium-sized flowers, as the style usually came into a position where pollen could fall on to it from the anthers. In the very small flowers of *E. scotica* the stigmas were not seen in such a position, but had doubtless been in it because there was pollen on them when the flowers opened. This interpretation conflicts with the statement of Müller, quoted on p.158, that in small flowers the style elongates after the flower opens, bringing the stigma below the anthers; it is, however, supported by Wettstein and by Schulz's observation that the stigma was beneath the anthers before the flowers opened in *E. minima* (p.158).

My observations show that there is a certain amount of individual variation in the development of the flowers, and it is probably on such minor variations that Schulz based his seven types of flower development.
The anthers were nearly always undehisced when the flower opened in *E. pseudokerneri* and sometimes in *E. anglica*. They were found to be dehisced when the flower opened in *E. brevipila*, *E. scotica* and *E. micrantha*, and usually in *E. confusa*. It was found that the anterior loculi dehisced before the posterior ones. The ease of successful emasculation varied from week to week in *E. micrantha* (E185A). This variation was again noted in *E. nemorosa* (E701) in 1957. In two plants which were starting to flower, several emasculations were attempted from 12 to 15 August. All were unsuccessful; if the flowers were taken young enough for the anthers to be undehisced, they were so small that emasculation became too difficult and the style was cut. From 20 August onwards, however, successful emasculation became possible, because the anthers were dehiscing at a later stage. Such variations may perhaps depend on water supply or temperature.

Pugsley (p.157) and most of the earlier authors held the view that the elongation of the corolla tube leads to self-pollination. However, the elongation of the corolla carries the anthers forward, thus increasing the length of style required to bring the stigma into a position beneath the anthers where pollen can fall on it. Therefore, elongation of the corolla will only cause self-pollination if the stigma is already held well down by the strong curvature of the style. If the stigma is above the lower edge of the anthers and quite close to them, a forward
movement by them can only bring the stigma into contact with their front or upper sides, away from the dehiscence lines.

The measurements of flower growth given for *E. pseudo-kerneri* indicate growth during most of the time the flower is open, and not merely until the anthers open as stated by Pugsley.

The abnormalities described include corolla-less flowers, which were also found by Heinricher (1898 b) in his cultures. He thought the condition was caused by aphides.

In large-flowered species in which the stigma does not quite reach beneath the anthers, my observations suggest that natural disturbance by wind, rain and animals may cause flowers to be self-pollinated. If this is so, flowers of these species are not entirely dependent upon insects for pollination.

**Insect Visitors to Flowers of Wild Euphrasia Plants.**

The following records of mine supplement those of previous workers given in Table 19, p.159:

**Diptera** (Determined by L. Parmenter):

(i) *Empis vitripennis* Mg., male (Empididae) at *E. brevipila* (E90), 22 June 1952. This small fly went well into the tube of the corolla, presumably for the nectar. It is so small that it did not appear to come into contact with the anthers and stigma.
(ii) *Platycerus albimanus* (Fab.), female (Syrphidae) at *E. nemorosa* (E344), 9 August 1953.

(iii) *P. manicatus* Mg., male (Syrphidae) at *E. pseudokerleri* (E594), 21 August 1955.

**Hymenoptera:**

(i) *Halictus fulvicornis* (Kirby), male (Apidae) at *E. pseudokerleri* (E23), 29 August 1951.

(ii) *H. tumulorum* (L.), or *perkinsi* Blüthgen, female (Apidae) at *E. nemorosa* (E401B), 19 August 1953.

*Halictus* visit the flowers for nectar; the head and thorax enter the throat of the corolla, and are likely to convey pollen.

**Insect Visitors to Flowers of Cultivated Euphrasia Plants.**

**Diptera** (Determined by L. Parmenter):

In the later part of the summer, flies habitually visit the flowers of *Euphrasia* cultivated in the greenhouse. These flies are usually Syrphidae, and may feed on nectar or pollen. Probably the individual species feed on one or the other and not on both. Flies feeding on nectar put their head and thorax into the mouth of the corolla. Those feeding on pollen 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. In 1957 flies, probably of the family Calliphoridae, became regular
visitors to the flowers; these flies appeared to be feeding on pollen, but instead of alighting regularly on the lower lips they crawled over the flowers in all directions.

In 1953 a large number of plants of *E. nemorosa* were grown on the experimental plot at University College, Leicester. The plants, of which 337 were present on 19 July, grew with extraordinary vigour, were profusely branched and bore many flowers. This patch of plants became much more of an attraction for insects than any wild colony of *Euphrasia* that I have seen. In warm weather insects were active at the flowers all the time.

Eight specimens of Syrphidae taken on 26 August, some feeding on pollen and some on nectar, have been identified as follows:-

- *Platycerius albimanus*, two females;
- *Sphaerophoria scripta* (L.), female;
- *Syrphus balteatus* (Deg.), male;
- *S. corollae* (Fab.), male;
- *S. luniger* Mg., female;
- *Eristalis arbustorum* (L.), female;
- *E. pertinax* (Scop.), female.

On 1 September another species of Syrphidae was taken, namely, *Scaeva pyrastris* (L.), male.

**Hymenoptera:**

I do not remember seeing any Hymenoptera visiting flowers of *Euphrasia* in the greenhouse, but they were seen at the flowers of *E. nemorosa* on the experimental plot in
1953, as follows:

29 and 30 July: Bombus sp. visiting flowers for a short time, and an Apis mellifera L. visiting them for long periods;

1 August: a Bombus lucorum (L.) visiting flowers for a short time; a small species of Halictus, with pollen on its back, went into one flower;

2 August: Bombus lucorum, _pratorum_ (L.) and Apis mellifera visiting flowers;

26 August: Bombus agrorum (Fab.), worker, taken at flowers;

27 August: Bombus lucorum, male, taken at flowers.

Discussion of Insect Visitors to Euphrasia Flowers.

All the Diptera, the two named Halictus species and Bombus lucorum have not previously been recorded at the flowers of Euphrasia. The fly Empis vitripennis (p.182) was behaving in the same way as Halictus minutissimus, observed by H. Müller, and the two insects are similar in size.

Müller mentions that the pollen is dry and powdery, and this is consistent with Wettstein's observation (p.155) that the pollen gets on to an insect by falling when the anthers are shaken. Inevitably some pollen falls on to the lower side of the corolla, and is usually to be found there when the anthers have dehisced. It was repeatedly noticed that pollen had fallen on to the yellow spot at the base of the lower lip. It was also noted that hoverflies (Syrphidae)
fed from this yellow spot. It seems possible that the function of the yellow spot is to heighten the visual effect of the patch of pollen which is usually present. Judging from the behaviour of insect 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.

Having considered the arrangements for pollination, we may now consider the occurrence of spontaneous hybrids, for the light they throw on the occurrence of cross-pollination in nature.

Wild hybrids may belong to the F1 generation, to the first backcross 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 F1 generation be recognized with certainty.

It has already been described how the flowers of the *E. nemorosa* populations grown in the garden at Leicester in 1953 became an attraction to insects. Six populations of *Euphrasia* were grown in rows, and the populations were well intermingled. Three populations were closely similar, and three others were all easily distinguishable by eye from the rest and from each other. The one which was distinct in most characters 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 there were about 303 plants on the plot, including only about 31 of El51A; because of this, and because El51A was a dwarf form which was less branched than the other populations, its flowers were greatly outnumbered by the others. The following year the progeny were grown in the garden by the same method as the parents; 79 "whalehide" pots, each with one Euphrasia and one plant of Plantago lanceolata, were planted out in the experimental plot at the University Botanic Garden, Cambridge, between 18 and 31 May. By 10 July it was clear that most of the Euphrasias were hybrids, but many of the dwarf, backward ones were apparently of the maternal type. The final score was 14 plants of the maternal type and 42 hybrids. That is, 75% of the survivors were hybrids. The hybrids, like most of their pollen parents, were very vigorous; the maternal type, however, 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%. 87 seedlings, including replacements, were planted out, so that if all those that died were of the maternal type the proportion of hybrids was 48%. Therefore, the true figure was not less than 48% and not more than 75%.

However, the following year about 200 more seeds of the original sample germinated. 82 seedlings were still
alive on 4 June 1955, and 30 were then potted up and grown in the greenhouse. There were five Euphrasias and two Plantago lanceolata seedlings in each pot. 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. The most plausible explanation is that the hybrid types germinated more readily than the maternal in the first year; another possible explanation would be that the maternal-type seedlings could survive longer in the seed pot before planting-out, which was carried out very late in 1955.

While this experiment cannot give a reliable estimate of the proportion of hybrids in the seed produced by the plants of El51A 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 (p.155). It seemed that the flowers of El51A were larger than they would have been in wild plants, and this was confirmed by a visit to its locality in 1954. It is not known whether increase in flower size induced by environment makes the flowers behave more like the habitually larger types of flower, but if it does the large flower-size obtained in the garden would have given a greater opportunity for out-crossing than occurs in most habitats in nature. In addition, the insect activity per flower was probably much
greater in the garden than it usually is in nature, in
spite of the large number of *Euphrasia* flowers present in
the garden. It is therefore likely that there was more
out-crossing in the garden experiment than there usually is
in nature.

Two hybrids were grown in pot cultures in 1954. In
1953 open-pollinated seed of *E. micrantha* (E185A) was
collected, but in view of the flower behaviour (pp.174-5)
it was not expected that this seed would produce any
hybrids. The uncounted seeds were sown late, on 17
February 1954, and only two germinated in that year. Both
plants were grown to maturity, and both proved to be
hybrids of *E. micrantha*. One had a yellow flower, and this
showed that its pollen parent was the yellow-flowered
*E. confusa* forma *confusa*, which had been grown in the
greenhouse with *E. micrantha* (E185A) the year before. In
the other plant, the flower was larger in size than that of
the lilac-flowered female parent and was white with a lilac
upper lip. It was probably a cross with *E. pseudokerneri*
which had also been grown with *E. micrantha* (E185A) in
1953. 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 suggests
that the hybrid types may germinate more readily than the
maternal; the results obtained with *E. nemorosa* (E151A) led
to a similar conclusion (p.188). 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.

The morphology of all the spontaneous cultivated hybrids will be dealt with later.

Three other unidentifiable *Euphrasia* plants have arisen from seed produced in the greenhouse, and these may have been hybrids, too.

The occurrence of wild hybrids can do no more than show that cross-fertilization does occur. Earlier workers on *Euphrasia* have recognized many hybrids. I have frequently found wild hybrids, and frequently met with them in herbaria. Of hybrids between species alike in chromosome number, I have found 11 dense populations extending over considerable areas, and 16 definite colonies of relatively small size. In addition, I have found 22 *Euphrasia* populations 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.

Large or very large populations of hybrids occur frequently when the parents are closely related, but there is also a case of a very large population of hybrids with parents in different subsections (*E. nemorosa* x *E. salisburgensis* var. *hibernica*). It must therefore be concluded
that any effects of a correlation between hybrid sterility and parental dissimilarity are liable to be modified by ecological conditions.

Since hybrids are almost always found when two species alike in chromosome number are very close together, it seems superfluous to list by name all the hybrids that have been found. The most frequently occurring hybrids in my collection are nemorosa x pseudokerneri (10 occurrences), confusa x occidentalis (5 occurrences) and confusa x micrantha (4 occurrences).

I have found four different pairs of species alike in chromosome number growing together without hybridization, at least in most of the area where they met. In two places on the Great Orme's Head I found E. curta and E. confusa growing together, and I could find no hybrids. In a third area there was hardly any mixing, nearly all of the plants being E. confusa, but one plant of E. curta and three plants of the hybrid were found. At the top of Lodge Hill, Box Hill, Surrey, E. nemorosa and E. pseudokerneri showed some separation of habitat, but in places were much mixed; here they grew largely without hybridization. In a few places there seemed to be hybrid forms, but they were not mixed with the parents or were accompanied by only one of the parent species. On the shores of Loch Rannoch, in some turfy patches interspersed with bare ground, I found E. micrantha and E. scotica growing together, and could not certainly identify any hybrids. In all these cases, the
species growing together belonged to the same series. The
next and last example concerns *E. confusa* and *E. micrantha*,
species in adjacent series of Pugsley's classification.
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.

**Discussion of the Occurrence of Hybrids.**

The regular occurrence of wild hybrids demonstrates
that insect-pollination is normally available for Euphrasias
in Great Britain; in the few cases where hybrids were
absent, at least locally, it is probable that ecological
conditions were unfavourable to hybrids.

It seems reasonable to infer from the observations on
both wild and spontaneous, cultivated hybrids that there is
in nature an appreciable gene flow over short distances in
most *Euphrasia* populations, including those of small-
flowered species.
In order to find out whether apomixis could occur, I cut the anthers and stigmas out of some flowers just before they opened. 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 attained 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, 2mm. 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 25mm. long or less. The number of flowers of each species for which this result was obtained is given in Table 21, p.194; 18 of the 35 flowers belonged to the diploid species, E. anglica and E. rivularis, and the rest to tetraploids. 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 2mm. long.

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

**TABLE 21: EUPHRASIA SPECIES TESTED FOR APOMIXIS**

<table>
<thead>
<tr>
<th>Euphrasia</th>
<th>Number of capsules of treated flowers failing to develop</th>
</tr>
</thead>
<tbody>
<tr>
<td>micrantha (E112A)</td>
<td>2</td>
</tr>
<tr>
<td>occidentalis (E73)</td>
<td>8</td>
</tr>
<tr>
<td>nemorosa (E74)</td>
<td>1</td>
</tr>
<tr>
<td>&quot; (E161)</td>
<td>1</td>
</tr>
<tr>
<td>pseudokerneri (E70)</td>
<td>1</td>
</tr>
<tr>
<td>&quot; (E77)</td>
<td>1</td>
</tr>
<tr>
<td>brevipila (E81)</td>
<td>3</td>
</tr>
<tr>
<td>anglica (E71)</td>
<td>12</td>
</tr>
<tr>
<td>&quot; (E76)</td>
<td>4</td>
</tr>
<tr>
<td>rivularis (E123)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>35</strong></td>
</tr>
</tbody>
</table>

**Self-fertility.**

Two types of bags were used for keeping insects from the flowers. The type used first was about 5cm. wide and 7 to 12 cm. long, and it was closed at the lower end with paper clips. There was space for growth in this bag, and it was left in position until all the space had been used. It tended to become flattened when closed. A smaller type
of bag (Fig. 65) was made soon after this and has been in use ever since. It was not fastened at the lower end and so did not become flattened. The lower end was filled with cotton wool which tended to keep the bag in position. These small bags were moved up as the stem grew, and could be taken off easily, so that pollination could be artificially assisted. Both types of bag were supported by wires stuck into the soil, and were made of grease-proof paper.

FIG. 65: Euphrasia plants with shoots covered to keep insects from the flowers, 1957.

Frequently the flowers were left to pollinate themselves, but pollination was sometimes assisted artificially by transferring pollen from anthers to stigma with a mounted needle. It was possible to see pollen grains on
the needle and on the stigma with a lens.

In order to extract the seeds from the capsule, a drop of water was introduced into it with forceps, and the seeds were then removed with forceps or a curved mounted needle. The seeds were held by the surface tension of the water, and were thus prevented from falling before being gripped by the forceps or in transit from the capsule to a safe place. Seeds were occasionally dropped, but the number lost was usually known. Sometimes it appeared that some seeds had been lost from the capsules when they were first found to have dehisced, and the number lost was then unknown. When the seed-production per capsule is given, therefore, capsules from which an unknown number of seeds was lost have been omitted, unless otherwise stated.

The figures for seed-production, resulting from the enforced self-pollination and from some open-pollination in the greenhouse, are given in Table 22, p.197. In 1952 the mean number of seeds per capsule was much lower in all the populations that were self-pollinated, except *E. occidentalis*, than in all the open-pollinated samples. This was probably due to interference with self-pollination caused by the flattening of the first type of selfing bag used. The better result in *E. occidentalis* may indicate that the leaves were large enough and stiff enough to protect the flowers from distortion by the bag. In later years such low figures were never obtained for self-pollination, the averages usually being as high as, or higher than, those for
TABLE 22: SEED PRODUCTION WITH ENFORCED SELF-POLLINATION
AND WITH OPEN-POLLINATION

<table>
<thead>
<tr>
<th>Year</th>
<th>Euphrasia</th>
<th>No. of seeds cap-</th>
<th>No. of good seeds per capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Self-pollinated</td>
<td>Bad</td>
<td>Good</td>
</tr>
<tr>
<td>1952</td>
<td>anglica (E71)</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nemorosa (E160)</td>
<td>-</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>occidentalis (E73)</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pseudokerneri (E42) &amp; (E70)</td>
<td>-</td>
<td>54</td>
</tr>
<tr>
<td>1953</td>
<td>anglica (E180)</td>
<td>3</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>occidentalis (E192)</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>salisburgensis (E230) var. hibernica</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>1954</td>
<td>pseudokerneri (E42) &amp; pseudokerneri (E226)</td>
<td>6</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1956</td>
<td>anglica (E616)</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>confusa (E601)</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hirtella (E559)</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>micrantha (E185A)</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nemorosa (E608)</td>
<td>4</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>occidentalis (E561)</td>
<td>6</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pseudokerneri (E609)</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>salisburgensis (E515)</td>
<td>46</td>
<td>4</td>
</tr>
<tr>
<td>1957</td>
<td>anglica (E663)</td>
<td>7</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nemorosa (E650)</td>
<td>13</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hirtella (E701)</td>
<td>2</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pseudokerneri (E699)</td>
<td>10</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open-pollinated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1952</td>
<td>anglica (E71)</td>
<td>-</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pseudokerneri (E42)</td>
<td>6</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1957</td>
<td>anglica (E663)</td>
<td>20</td>
<td>24</td>
</tr>
</tbody>
</table>

+ = pollination assisted in some or all of the flowers.
open-pollination. The highest maximum figure for self-pollination is 21 seeds per capsule; the lowest maximum after 1952 is 8, but if only samples with 6 or more capsules are counted the lowest maximum after 1952 is 12.

Nearly all the bad seeds of self-pollinated flowers in Table 22 were very small empty ones, similar to those produced by an unpollinated capsule described on p.193. Two were large empty ones, and a few were undersized with much reduced contents.

One sample of *E. anglica* (E76), when open-pollinated in 1952, produced eight bad seeds; six are accounted for in Table 22, and there were two more in capsules from which an unknown number of seeds was lost. The bad seeds consisted of full-sized but empty testas; all were produced by capsules which also contained normal seeds. This type of bad seed is the main type of seed resulting from the pollination of diploids by tetraploids; in addition, it has been found in hybrids and, very rarely, when species have been self-pollinated. The large empty seeds produced by this open-pollinated diploid were probably due to cross-pollination with tetraploids.

Empty seeds were again found in open-pollinated *E. anglica* (E663) in 1957; 19 of the 20 bad seeds were of this type. The seeds in the four capsules concerned were as follows:

- capsule 1 - 2 good, 9 large empty;
- capsule 2 - 3 good, 9 large empty;
- capsule 3 - 10 good, 1 large and 1 small empty;
- capsule 4 - 9 good.
It seems probable that the two flowers with 9 large empty seeds were pollinated soon after they opened by insects carrying pollen from tetraploids.

Capsules with very few seeds are sometimes undersized, and their dehiscence is probably somewhat retarded.

It is evident from these results that enforced self-pollination usually gives a full yield of seed, but there is no evidence to show whether or not the species listed differ in seed-production per capsule.

The germination of seed produced by self-pollination is shown in Table 23. As has been mentioned, unexpectedly

### Table 23: Germination of Seed Produced by Self-Pollination

<table>
<thead>
<tr>
<th>Date sown</th>
<th>No. sown</th>
<th>No. germinating at spring</th>
<th>No. germinating 2nd spring</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1952</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica (E71)</td>
<td>20/12/52</td>
<td>55</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>nemorosa (E60)</td>
<td>&quot;</td>
<td>40</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>occidentalis (E73)</td>
<td>&quot;</td>
<td>47</td>
<td>not known (15)</td>
<td></td>
</tr>
<tr>
<td>pseudokerneri (E42) &amp; (E70)</td>
<td>&quot;</td>
<td>65</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td><strong>1953</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica (E180)</td>
<td>15/2/54</td>
<td>97</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>occidentalis (E192)</td>
<td>&quot;</td>
<td>34</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>salisburgensis var. hibernica (E230)</td>
<td>&quot;</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>1954</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pseudokerneri (E226)</td>
<td>20/1/55</td>
<td>99</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>1956</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica (E616)</td>
<td>14/12/56</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>confusa (E601)</td>
<td>&quot;</td>
<td>31</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>hirtella (E559)</td>
<td>&quot;</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>micrantha (E185A)</td>
<td>&quot;</td>
<td>28</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>nemorosa (E608)</td>
<td>&quot;</td>
<td>81</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>occidentalis (E561)</td>
<td>&quot;</td>
<td>84</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pseudokerneri (E609)</td>
<td>&quot;</td>
<td>49</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>
poor germination sometimes occurs, so that one cannot draw any special conclusions from Table 23. However, there is some suggestion that the viability is lower than that of wild seed.

IV. Cross-Fertility

Methods.

The flowers were protected from insects by the smaller of the two types of bag described earlier. The anthers were removed by cutting the filaments (Fig. 59, p.154) from below with a small pair of curved nail scissors. In large-flowered forms this was sometimes done as the flower was opening, but it was preferable to do it the day before the flowers were due to open, and occasionally it was necessary to do it earlier than this. If the flower was shut, emasculation involved making cuts in the corolla, but this had no ill-effects. The anthers were drawn out of the corolla with a mounted needle and, if all the filaments were cut, they came out as a block. Frequently, however, not all the filaments were cut, some anthers remained behind, and more cuts had to be made. The anthers were transferred to a slide and examined with a lens. If they were dehisced, or broken during extraction, the flower could still be used for cross-pollination provided there appeared to be little or no pollen on the stigma when examined with a lens.
The stigma was pollinated at the time of emasculation or up to two or three days after the flower opened. Pollen was transferred to the stigma on a mounted needle, and a lens was used to see that the stigma was well-pollinated. For preference, pollen was collected from the anthers of newly-opened flowers, to minimize the chance of introducing foreign pollen brought to the flowers by insects. Sometimes it was necessary to use old flowers and to take pollen from the mouth of the corolla tube.

The seed of flowers in which the anthers were unopened at the time of emasculation was kept separate from the seed of flowers in which the anthers were dehisced or broken, in case some of this had resulted from self-pollination.

**Parents Differing in Chromosome Number.**

In August 1952, I began making crosses between species alike in chromosome number, and between species differing in chromosome number, counting the chromosomes when necessary.

The results of crosses between species differing in chromosome number are given in Table 24, p.202. This includes figures for capsules where an unknown number of seeds was lost. There are, however, few of these because bad seeds, which predominated, do not fall out of the capsules as easily as good ones.

Where the diploid was the female parent, and there was no selfing, only one or two good seeds were produced among 351 bad ones, and the number of bad seeds produced was
TABLE 24: SEED PRODUCTION FROM CROSS-POLLINATION OF SPECIES DIFFERING IN CHROMOSOME NUMBER

<table>
<thead>
<tr>
<th>Euphrasia</th>
<th>Selfing impossible</th>
<th>Selfing possible</th>
<th>No. of capsules</th>
<th>No. of seeds</th>
<th>No. of capsules</th>
<th>No. of seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid female x Tetraploid male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1952</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica x brevipila (E76 x E81)</td>
<td>1</td>
<td>13</td>
<td>0</td>
<td>3</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>&quot; x scotica (E76 x E120)</td>
<td>1</td>
<td>18</td>
<td>0</td>
<td>3</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x confusa (E76 x E179)</td>
<td>1</td>
<td>13</td>
<td>0</td>
<td>2</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>&quot; x &quot; (E76 x E209)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x pseudokerneri (E76 x E77)</td>
<td>2</td>
<td>25</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x nemorosa (E76 x E154)</td>
<td>1</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x &quot; (E168 x E154)</td>
<td>3</td>
<td>18</td>
<td>0</td>
<td>4</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x pseudokerneri (E168 x E77)</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x nemorosa (E135 x E196)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x &quot; (E135 x E154)</td>
<td>1</td>
<td>16</td>
<td>0</td>
<td>8</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td>&quot; x confusa (E135 x E179)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>41</td>
<td>2</td>
</tr>
<tr>
<td>1953</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica x nemorosa (E180 x E196)</td>
<td>12</td>
<td>151</td>
<td>1 or 2</td>
<td>2</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>&quot; x micrantha (E180 x E185A)</td>
<td>7</td>
<td>79</td>
<td>0</td>
<td>3</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>TOTALS</td>
<td>30</td>
<td>351</td>
<td>1 or 2</td>
<td>40</td>
<td>311</td>
<td>47</td>
</tr>
</tbody>
</table>

(Mean per capsule = 11.7) (Mean per capsule = 9)

Tetraploid female x Diploid male

| 1952 | | | | | | |
| pseudokerneri x anglica (E42 x E76) | 1 | 0 | 0 | - | - | - |
| " x " (E77 x E76) | - | - | - | 2 | 0 | 0 |
| nemorosa x anglica (E129 x E76) | - | - | - | 5 | 0 | 0 |
| 1953 | | | | | | |
| nemorosa x anglica (E196 x E180) | 1 | 11 | 0 | - | - | - |
| (2) | 1 | 7 | 0 | - | - | - |
| 1955 | | | | | | |
| nemorosa x rostkoviana (E474 x E520) | - | - | - | 2 | 10 | 3 |
| " x anglica (E474 x E493) | 10 | 14 | 0 | 5 | 10 | 0 |
| micrantha x " (E185A x E493) | 2 | 16 | 0 | - | - | - |
similar to the number of good seeds produced by open-pollination or selfing. All the bad seeds of the diploid female parent consisted of full-sized testas with little or no contents. Capsules containing them developed normally and dehisced, though it sometimes seemed as if their dehiscence was abnormally retarded.

With the tetraploid as female, full-sized empty seeds were not produced. Some of the 11 seeds of *E. nemorosa* (E196) x *E. anglica* (E180) were very nearly empty, and some were partly filled; however, they were not thought to be worth sowing. In the cross between *E. nemorosa* (E474) and *E. anglica* (E493), the seeds included in Table 24, which were produced in 3 out of the 15 capsules, were considerably undersized, but were fairly full. There was probably some development of the capsules. The two capsules of *E. micrantha* (E185A) x *E. anglica* (E493) were both partially developed; in one there were six rather small and nearly empty seeds, and in the other there were five small and nearly empty seeds and five with contents amounting to about a quarter of the bulk of a normal seed. A similar seed was produced by *E. nemorosa* (E474) x *E. rostockiana* (E520) in a partially selfed capsule that also yielded three normal seeds.

All the other capsules in crosses with tetraploids as female were more or less undeveloped, and doubtless contained minute empty ovules, which were seen in a number of cases though not usually counted.
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 in 1952 the reverse type of cross seemed more promising.

Parents Alike in Chromosome Number.

The seed-production of crosses of this type is given in Table 25, p.205. It will be noted that two intraspecific crosses between different populations of *E. anglica* were made in 1952, and another was made in 1957; in all three cases the parents were morphologically distinguishable from each other.

A number of capsules failed to develop, and are not included in the table; it was not usually known why they failed to develop, but in some cases the stigma was brown at the time of pollination, and so presumably not functioning; the browning of the stigma might have been caused by damage to the style during emasculation.
TABLE 25: SEED PRODUCTION FROM CROSS-POLLINATION BETWEEN FORMS ALIKE IN CHROMOSOME NUMBER

| Euphrasias (female x male) | Selfing impossible | | Selfing possible | |
|---------------------------|---------------------|---------------------|---------------------|
|                           | No. of caps | No. of seeds | Bad | Good | No. of caps | No. of seeds | Bad | Good |
| 1952 | anglica x anglica (E76 x E71) | - | - | - | 1 | 0 | 13 | |
| | " x " (E76 x E168) | 2 | 0 | 38 | 1 | 0 | 16 | |
| | pseudokerneri x nemorosa (E42 x E154) | 2 | 0 | 13 | 2 | 0 | 15 | |
| | " x brevipila (E70 x E81) | - | - | - | 1 | 0 | 5 | |
| | brevipila x scotica (E81 x E120) | - | - | - | 3 | 3 | 20 | |
| | pseudokerneri x scotica (E42 x E120) | 1 | 0 | 8 | 1 | 0 | 11 | |
| 1953 | pseudokerneri x occidentalis (E226 x E192) | 2 | 1 | 7+ | 4 | 0 | 15+ | |
| | occidentalis x salisburyensis var. hibernica (E192 x E230) | 6 | 4 | 42+ | 4 | 2 | 24 | |
| | salisburyensis var. hibernica x occidentalis (E230 x E192) | 7 | 7 | 28+ | 3 | 4 | 19 | |
| 1956 | anglica x hirtella (E616 x E559) | 4 | 1 | 7+ | 4 | 0 | 15+ | |
| | pseudokerneri x nemorosa (E609 x E608) | 3 | 5 | 36 | - | - | - | |
| | " x brevipila (E609 x E623) | 1 | 0 | 10 | - | - | - | |
| | " x micrantha (E609 x E185A) | 4 | 4 | 49 | - | - | - | |
| | nemorosa x salisburyensis (E608 x E515) | 1 | 1 | 11 | 2 | 1 | 20 | |
| | salisburyensis x nemorosa (E515 x E608) | - | - | - | 2 | 4 | 22 | |
| 1957 | anglica x anglica (E663 x E649) | 5 | 4 | 28+ | 3 | 6 | 36 | |
| | pseudokerneri x micrantha (E699 x E185A) | 4 | 8 | 34+ | 1 | 6 | 8 | |

TOTALS: 42 35 311 32 26 239

(Mean per capsule = 8.2) (Mean per capsule = 8.3)

+ = Some other seeds lost, or probably lost.
Most of the bad seeds that were produced were the minute empty ones described previously (p.193), but in the cross *E. occidentalis* x *E. salisburgensis* var. *hibernica* in 1953 two of the bad seeds were half filled and large; in the similar cross *E. nemorosa* x *E. salisburgensis* in 1956 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 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* x *E. anglica* in 1957, four of the total of ten bad seeds were large empty ones. In the cross *E. pseudokerneri* x *E. micrantha* in 1957 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.

In the table the species pairs are arranged in order of increasing dissimilarity in each year. The smallest number of good seeds in one capsule was two, and the largest, in *E. anglica* x *E. anglica* (E76 x E168), twenty-one. The largest number in an interspecific cross was 13, which occurred both in *E. pseudokerneri* x *E. micrantha* and in *E. salisburgensis* x *E. nemorosa* in 1956.

These results may be summed up by saying that these cross-pollinations produced chiefly good seed. Also, the yield of seed per capsule was below the average yield of
seed in the diploid female x tetraploid male crosses (Table 24, p.202), and about in the middle of the range for open-pollinated and self-pollinated seed production (Table 22, p.197). A few bad seeds were produced, which were usually small and empty but were occasionally large and empty or partly-filled. Apart from some large empty ones found rather surprisingly in an intraspecific cross, large and empty or partly-filled seeds were found only in crosses of *E. salisburgensis* with tetraploids of subsection Ciliatae - the widest crosses made; in two of these crosses some of the "good" seeds were slightly undersized. Thus, in the production of seeds there is some evidence of inviability after fertilization in the widest crosses; small empty seeds are probably the result of failure of pollination.

**Germination of Hybrid Seed.**

The germination of seed from cross-pollinated flowers is given in Table 26, p.208. The seed-pots were thrown away after the first year of germination, except for the 1956 pots, all of which were kept until 1958 and, in some cases, gave rise to seedlings in two seasons. In the cross *E. occidentalis* x *E. salisburgensis* var. *hibernica* in 1953 the seedlings died early, but it was noted that in sample (1) they recalled *E. salisburgensis* and were probably hybrids, and that in sample (2) the one seedling looked like *E. occidentalis*. 
### TABLE 26: GERMINATION OF HYBRID SEED, AND PLANTS RAISED FROM IT

<table>
<thead>
<tr>
<th>Euphrasias (female x male)</th>
<th>No. of seeds sown</th>
<th>No. of seedlings</th>
<th>Plants raised hybrid</th>
<th>Plants raised non-hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1952</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica x anglica (E76 x E71)</td>
<td>13*</td>
<td>9</td>
<td>6 + 1?</td>
<td></td>
</tr>
<tr>
<td>&quot; x &quot; (E76 x E168)(1)</td>
<td>38</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>&quot; x &quot; (E76 x E168)(2)</td>
<td>16*</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>pseudokerner x nemorosa (E42 x E154)(1)</td>
<td>15*</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>&quot; x &quot; (E42 x E154)(2)</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&quot; x brevipila (E70 x E81)</td>
<td>4*</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>&quot; x scotica (E42 x E120)</td>
<td>18*</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>brevipila x scotica (E81 x E120)</td>
<td>22*</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>anglica x brevipila (E76 x E81)</td>
<td>6*</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>&quot; x pseudokerner (E68 x E77)</td>
<td>11*</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>&quot; x confusa (E76 x E179)</td>
<td>2*</td>
<td>0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>&quot; x &quot; (E135 x E179)</td>
<td>2*</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>&quot; x nemorosa (E135 x E196)</td>
<td>10*</td>
<td>4</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>&quot; x &quot; (E135 x E154)</td>
<td>27*</td>
<td>6</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><strong>1953</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pseudokerner x occidentalis (E226 x E192)</td>
<td>25*</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>salisburgensis var. hibernica x occidentalis (E230 x E192)</td>
<td>46*</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>occidentalis x salisburgensis var. hibernica (E192 x E230)(1)</td>
<td>41</td>
<td>4</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>occidentalis x salisburgensis var. hibernica (E192 x E230)(2)</td>
<td>17*</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>nemorosa x anglica (E196 x E180)</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>anglica x nemorosa (E180 x E196)</td>
<td>2</td>
<td>0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>1956</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anglica x hirtella (E616 x E559)</td>
<td>24</td>
<td>2</td>
<td>1 + 1?</td>
<td></td>
</tr>
<tr>
<td>pseudokerner x nemorosa (E609 x E608)</td>
<td>27</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&quot; x brevipila (E609 x E623)</td>
<td>10</td>
<td>6</td>
<td>3?</td>
<td></td>
</tr>
<tr>
<td>&quot; x micrantha (E609 x E185A)</td>
<td>45</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>nemorosa x salisburgensis (E608 x E515)</td>
<td>32*</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>salisburgensis x nemorosa (E515 x E608)</td>
<td>22*</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* = May include some seeds resulting from self-pollination.
The table shows that where the parents' chromosome numbers were the same nearly all the plants raised were hybrids, and where they differed no hybrids were raised. All the non-hybrid plants were like the seed-parents, and presumably resulted from accidental self-pollination. No such plants occurred where it had been judged that there was no possibility of selfing. It is tantalizing that the seedling of *E. nemorosa* x *E. anglica* (1953) died, for it would have been a hybrid from parents differing in chromosome number, provided it had been correctly assumed that there had been no chance of self-pollination.

Some indication of the frequency of wild hybrids between species alike in chromosome number has been given earlier (pp.190-192). It is not surprising, therefore, that cross-pollinations in cultivation should lead to the production of hybrids both by spontaneous crossing (pp.186-190) and by artificial crossing (Table 26, p.208). That the cultivated plants concerned were in fact hybrids is borne out by the descriptions that follow.

V. Artificial Hybrids in *Euphrasia*

**Morphology.**

Most of the description is pictorial, and the illustrations are photographs of:-(1)herbarium specimens, (2)leaf shapes, and (3)flower shapes. The leaf and flower shapes are obtained by photographic contact printing from specimens
mounted according to a method described to me by Mr. D. A. Wilkins of the Scottish Plant Breeding Institute. The specimens are stuck to cellulose adhesive tape which is then stuck to glass. The tape is conveniently mounted on lantern-plate cover-glasses, 3\(\frac{1}{4}\) inches square (Fig. 66). A partly filled strip of tape can always be peeled off the glass for the addition of more specimens. When the flowers are dry they keep their condition for some months, and often for years. Leaves mounted in this way tend to crack after some years, and flowers may crack or become translucent. Cool conditions of storage seem to be best. Contact prints provide a permanent record, and a negative print of reasonable quality gives very good positive prints by being used as a transparency in contact printing.

FIG. 66: A slide with leaves mounted on cellulose tape and labelled. Positive made from contact negative.
In the following account, the name of the female parent is given first in all hybrid formulae, and unless otherwise stated all the plants mentioned were grown in the greenhouse with *Medicago lupulina* as host plant.

The first group of hybrids to be described is that in which crosses were made between different populations of *E. anglica*.

**E. anglica x E. anglica (E76 x E168) and (E76 x E71):**

In 1952, one plant from Box Hill (E76) was pollinated from one or more Charnwood Forest plants (E168), and a second was pollinated from one or more New Forest plants (E71). The hybrids were grown in 1954. In that year, in another cross, a plant of the Box Hill form was obtained through accidental selfing of a third Box Hill plant (E76). Five Charnwood Forest plants were also obtained in 1954 through accidental selfing in another attempted cross. The New Forest plants were self-pollinated, and six plants were grown in 1954. Another one occurred as a stray in another seed-sample.

The herbarium photograph (Fig. 67, p.212) shows a selection of the available plants. There is no Box Hill plant (E76), because the main stem of the 1954 plant was diseased, and the 1952 plants were also unsuitable for purposes of comparison owing to different conditions of cultivation. The photograph includes six of the 19 hybrids between the Charnwood and the Box Hill plants; the four that were pressed in August are suitable for comparison
Charnwood (E168)
5 plants: pressed 21/8/54.

Box Hill (E76) x Charnwood (E168)
6 plants: 2 left pressed 12/8/54;
2 lower right pressed 22/8/54;
2 upper right pressed 10/7/54.

Top row: New Forest (E71)
3 plants: pressed 6/8/54.
Bottom row: Box Hill (E76) x New Forest (E71)
3 plants: pressed 10/7/54.

FIG. 67: Herbarium specimens of hybrids and their parents (about 1/4 natural size): all E. anglica.
with the five Charnwood plants. From the latter they differ in being larger and in having larger leaves; the branches tend to be shorter in relation to the main stem. The three New Forest plants (E71) have large leaves, short internodes and rather few, erect branches. The hybrids between the New Forest and the Box Hill plants have larger leaves still and longer cauline internodes; the branches are similarly few, but are more spreading. These hybrids may also be compared with the two plants of the other hybrid (E76 x E168) that were pressed on the same day (10 July); they have rather larger leaves, fewer branches, and had produced more flowers by this date.

The node of flowering and number of branches are shown for both crosses in Table 27. Here, and throughout the account of hybrid morphology, the cotyledonary node is excluded in counting nodes. Filiform branches under about

| TABLE 27: NODE OF FIRST FLOWER (F) AND NUMBER OF PRIMARY BRANCHING NODES (B) OF E. ANGLICA HYBRIDS AND PARENTS |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Number of plants                                | New Forest (E71)                                 | E76 x E71                                       | E76 x E168                                      | Charnwood (E168)                                 |
| Mean of F                                       | 5.3                                             | 5.2                                             | 6.9                                             | 6.8                                             |
| Range of F                                      | 4 - 7                                           | 5 - 6                                           | 4 - 9                                           | 5 - 8                                           |
| Mean of B                                       | 2.1                                             | 2.3                                             | 2.7                                             | 3.0                                             |
| Range of B                                      | 1 - 3                                           | 1 - 4                                           | 1 - 5                                           | 2 - 4                                           |
2 cm. long were not counted. The table shows that the New Forest form and its hybrid have lower flowering nodes and fewer branches than the Charnwood form and its hybrid.

Leaf silhouettes are shown in Fig. 68. A few leaves

Node: 9 10 9 9 9
Charnwood (E168)

Box Hill (E76) x Charnwood (E168). All leaves from node 9.

Node: 8 9
Box Hill (E76). One plant.

Box Hill (E76) x New Forest (E71). Mounted dry. All leaves from node 9.

New Forest (E71). All leaves from node 10.

FIG. 68: Leaves of hybrids and parents: \textit{E. anglica}. Each leaf taken from a different plant, except in E76.
that were dried between papers before being mounted on the cellulose tape are included; they have probably not shrunk much. The leaves of the Box Hill plant have coarser teeth than those of the Charnwood plants and are broad across the top pair of teeth, while the leaves of the Charnwood plants taper evenly towards the apex; the leaves of their hybrids show a range in both characters from one parental extreme to the other. The leaves of the New Forest plants (E71) are broader and more rounded in outline than those of the Box Hill plant (E76); those of their hybrids tend to be broad, as in E71, and truncate at the base, as in E76.

The flowers of this group of hybrids are shown in Fig. 69, p.216. In the centre are the flowers of the one plant of the Box Hill population that was available in 1954. They are rather large, and all their parts are broad. As is commonly the case in E. anglica there is little dilation of the lobes towards the apex. The flowers of all five Charnwood plants are shown at the top left. Slight differences can be made out between the flowers of individual plants. The flowers are smaller than those of the Box Hill plant, and the lobes of the lower lip are narrower; the length of the midlobe is variable. Flowers of ten plants of the hybrid between these two are shown. The plants show individual variation in flower size and shape; they can be seen to be intermediate between those of the parents. At the top right of Fig. 69 are the flowers of the New Forest form (E71). They are a little smaller.
FIG. 69 FLOWERS OF HYBRIDS AND THEIR PARENTS: ALL E. ANGLICA.
FLOWERS OF EACH PLANT BRACKETED TOGETHER

- E168 CHARNWOOD
- E76 BOX HILL
- E71 NEW FOREST

E76 x E168
E76 x E71

PLANT 8
PLANT 7
PLANT 7?
than the Box Hill flowers (E76), with narrower lobes and a distinctly dilated and rather long midlobe. Those of the hybrid (E76 x E71) vary in size; in shape they tend to have broad lobes and a dilated midlobe, and sometimes dilated side lobes.

In the account of the next group of hybrids all aspects of each hybrid will be dealt with together.

**E. pseudokernerii** x **E. nemorosa** (E42 x E154):

One plant of this hybrid, from the seed sample in which there was no chance of selfing, was grown in 1953. It was very vigorous and inconveniently large for including in a herbarium photograph. In 1954 three plants were grown from the seed sample which might possibly have included selfed seeds. One of the plants, which may or may not have been a hybrid, was highly abnormal with fleshy, almost linear leaves. Similar symptoms have been met with in another population (not a hybrid), which suggests that the abnormality was due to disease. The other two plants (eventually determined as hybrids) are the upper ones in Fig. 70, p.218. The two lower plants in Fig. 70 are **E. nemorosa** (E419, a later gathering from the locality of E154). The other parent, **E. pseudokernerii** (E70, from the same locality as E42), is shown in Fig. 71, p.219. Though the hybrids are more robust than **E. nemorosa** and have more erect branches than **E. pseudokernerii**, they do not appear to be intermediate between the parents, which in any case are very similar to one another. However, the leaf teeth of
FIG. 70: Euphrasia hybrid and one parent (about 1/2 natural size). Top row - E. pseudokernerri x E. nemorosa (E42 x E154); pressed 22/8/54. Bottom row - E. nemorosa (E419, from locality of E154); pressed 17/9/54.

The hybrids are sharper than those at the same node (the thirteenth) in E. nemorosa, and blunter and shallower than those of E. pseudokernerri at the fourteenth node (Fig. 72, p.220); therefore, in this respect, they are intermediate between the parents, but there is little difference in leaf outline.

The flowers of E. pseudokernerri x E. nemorosa can be compared with those of the two parents in Fig. 73, p.221. E. nemorosa (E419) differs from E. pseudokernerri (E70 and E42) in its smaller flowers with narrow, less divergent
Top row: *E. pseudokernerii* (E70, from locality of E42)
2 plants: pressed 9/9/54.

Bottom rows: *E. pseudokernerii* x *E. brevipilu* (E70 x E81)
3 plants: 1 upper left pressed 6/8/54; 1 lower left pressed 1/9/54; 1 right pressed 6/8/54.

Top row: *E. pseudokernerii* x *E. scotica* (E42 x E120)
4 plants: 1 left pressed 28/8/54; 3 right pressed 5/8/54.

Bottom row: Left - *E. brevipilu* x *E. scotica* (E81 x E120)
3 plants: pressed 22/7/54.

Right - *E. scotica* (E659, from locality of E120)
4 plants: pressed 21/8/56.

FIG. 71: *Euphrasia* hybrids and some of their parents (about ¼ natural size).
E. nemorosa (E419, from locality of El54). All leaves from node 13.

E. pseudokerneri x E. nemorosa (E42 x El54). All leaves from node 13.

E. pseudokerneri (E70). All leaves from node 14. (From the same locality as E42).

E. pseudokerneri x E. brevipila (E70 x E81). All leaves from node 11.

E. pseudokerneri x E. scotica (E42 x El20). All leaves from node 11.

Node: 9 10 10

E. brevipila x E. scotica (E81 x El20). Mounted dry.

FIG. 72: Leaves of Euphrasia hybrids and some of their parents. Each leaf taken from a different plant.
FIG. 73 FLOWERS OF HYBRIDS AND THEIR PARENTS
FLOWERS OF EACH PLANT BRACKETED TOGETHER
lobes; the midlobes in some individuals are dilated apically. The hybrid flowers are as small as those of the pollen parent (*E. nemorosa*) but have slightly broader and slightly more divergent lobes. The flowers and the leaf dentition provide the chief evidence that two plants of the hybrid between *E. pseudokernerli* x *E. nemorosa* were obtained.

**E. pseudokernerli** x **E. brevipila** (*E70 x E81*):

There was a possibility of selfing in this seed sample but the plants raised were hybrids. The only specimens of *E. brevipila* available were grown in their own turf in 1952 and are not comparable with plants cultivated in 1954. Fig. 71, p.219, shows that the hybrids differed from *E. pseudokernerli* in their more ascending branches; they also flowered earlier, as can be seen from the fact that they are evidently more advanced in development than either of the *E. pseudokernerli* plants shown, although two of the hybrids were pressed much earlier.

The leaves of *E. pseudokernerli** x **E. brevipila* in Fig. 72, p.220, are extremely similar to those of *E. pseudokernerli*, although they were taken from three nodes lower. The transition in leaf shape up the stem takes place more rapidly in those plants which flower at an earlier node, and the leaves at node 11 in this hybrid are probably equivalent, in terms of maturity of form, to those at node 14 in the later-flowering *E. pseudokernerli*.

As is usual in *E. brevipila*, the leaves of E81 had short-stalked glands, as well as some bristles. Two plants
of *E. pseudokerneri* x *E. brevipila* were slightly glandular and bristly, while the third was eglandular and less bristly. *E. pseudokerneri* is normally considered to be eglandular, but a few glands were found on some of the leaves of the three best-developed plants of E70; there were also a few bristles. However, the glands were more numerous than this on the two glandular plants of the hybrid.

This hybrid flowered at a much lower node than *E. pseudokerneri* (Table 28). Its flowers were very similar to those of *E. pseudokerneri*, but the lateral lobes curved outwards (Fig. 73, p.221). The flowers of *E. brevipila*, grown two years earlier, were smaller, being about 2mm. narrower and about 2.5mm. shorter.

**TABLE 28: NODE OF FIRST FLOWER (F) AND NUMBER OF PRIMARY BRANCHING NODES (B) OF EUPHRASIA HYBRIDS AND PARENTS**

<table>
<thead>
<tr>
<th></th>
<th><em>E. pseudokerneri</em></th>
<th><em>E. pseudokerneri</em> x <em>E. brevipila</em></th>
<th><em>E. pseudokerneri</em> x <em>E. scotica</em></th>
<th><em>E. brevipila</em> x <em>E. scotica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of plants</td>
<td>5</td>
<td>3</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Mean of F</td>
<td>14.6</td>
<td>8</td>
<td>8.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Range of F</td>
<td>13 - 16</td>
<td>7 - 9</td>
<td>7 - 9</td>
<td>6 - 7</td>
</tr>
<tr>
<td>Mean of B</td>
<td>5.4</td>
<td>4.3</td>
<td>2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Range of B</td>
<td>3 - 10</td>
<td>3 - 5</td>
<td>2 - 4</td>
<td>0 - 1</td>
</tr>
</tbody>
</table>
E. pseudokerneri x E. scotica (E42 x E120):

There was a slight possibility of selfing in this seed-sample, but the ten plants raised were clearly hybrids. There are no specimens of E. scotica (E120), but Fig. 71, p.219, and Fig. 73, p.221, show wild plants and flowers of this species (E659) collected within 20 yards of the place where E120 had been collected four years previously. The two species, E. pseudokerneri and E. scotica, show in almost every way the greatest dissimilarity possible among tetraploid species within Subsection Ciliatae. E. pseudokerneri is much-branched, late-flowering and large-flowered, and it inhabits dry chalk grassland in England, where it is endemic. E. scotica is slender, sparingly branched, rather early-flowering and very small-flowered, and it inhabits wet flushes in the mountains of the British Isles, Faroe and Norway. Fig. 71, p.219, shows that the hybrid was intermediate between the parents in habit. Compared with E. pseudokerneri, the hybrid began flowering at a lower node, and it had on the average fewer branches (Table 28, p.223) and shorter and broader leaves, that were broadly cuneate at the base, instead of truncate, and had fewer teeth (Fig. 72, p.220). The difference in the number of teeth is statistically significant (P = .05). The indumentum of two of the hybrid plants was noted; they were similar to their parents in being eglandular and almost without bristles.

The flowers of E. scotica (E120), one of which is
shown in Fig. 64, p.172, and the flowers of *E. scotica* (E659) (Fig. 73, p.221) had the lobes of the lower lip shallowly emarginate and the midlobe parallel-sided or slightly dilated. In the rather larger flowers of the hybrid (Fig. 73) the lobes of the lower lip were more deeply emarginate and the midlobe was more dilated; these hybrid flowers were thus intermediate between those of the parents.

*E. brevipila x E. scotica* (E81 x E120):

There was almost certainly selfing in the production of this seed sample, but the three plants raised were hybrids; they are shown in Fig. 71, p.219, and are almost unbranched. Probably the natural state of both the parent populations is to be sparingly branched; if so, the hybrid is not similar to the parents in this character. The node of the first flower was lower for this hybrid than for *E. pseudokerneri x E. scotica* (Table 28, p.223); this was to be expected, as both *E. brevipila* and *E. scotica* are early-flowering, whereas *E. pseudokerneri* is not. The number of leaf teeth seems to be smaller, but the leaf shape is very similar to that of *E. pseudokerneri x E. scotica* (Fig. 72, p.220). Two plants of *E. brevipila x E. scotica* had freely glandular leaves, and the other had slightly glandular leaves; all had a few bristles. They were, therefore, more strongly glandular than *E. pseudokerneri x E. brevipila*.

The smaller flower-size of *E. brevipila* (described on
p.223) compared with *E. pseudokernerri* seems to have caused *E. brevipila* x *E. scotica* to have smaller flowers than *E. pseudokernerri* x *E. scotica* (Fig. 73, p.221). The shape of the lower lip in *E. brevipila* x *E. scotica* is more like that of *E. scotica* than it is in *E. pseudokernerri* x *E. scotica*.

The next three hybrids to be discussed derive from one or both of *E. occidentalis* and *E. salisburgensis*.

*E. pseudokernerri* x *E. occidentalis* (E226 x E192):

In 1953, two plants of *E. pseudokernerri* (E226) were used for artificial hybridization with *E. occidentalis*. One (grown on *Anthyllis vulneraria*) flowered late after producing many branches, and is shown in Fig. 74, p.227; the other flowered earlier and had fewer branches. Fig. 74 also shows three plants of *E. occidentalis* (E192); the middle one was grown on *A. vulneraria*, and the right-hand one was obtained in 1955 by selfing a plant grown in 1953. These photographs show the extreme difference in habit between *E. pseudokernerri* and *E. occidentalis*, and the intermediate habit and leaf size of their F1 hybrids. In the production of the seed-samples from which these hybrids grew, there was a possibility of selfing and of open pollination, because the bags fell off the flowers. However, all plants appeared to be the intended hybrid. The three plants of the F1 hybrid shown in Fig. 74 were the only ones grown in 1954, but another was grown in 1955.

An F2 generation was raised, and five of the eight
Top row: Left - E. pseudokernerii (E226)
1 plant: pressed 10/9/53.
Right - Hybrid F 1
1 plant: pressed 4/10/54.
Middle row: E. occidentalis (E192)
3 plants: 1 left pressed 2/9/53; 1 centre pressed 24/9/53; 1 right pressed 1/9/55.
Bottom row: Hybrid F 1
2 plants: pressed 9/9/54.

Top row: Hybrid F 2
2 plants: pressed 9/8/55.
Bottom rows: Hybrid F 2
3 plants: 1 upper left pressed 18/9/56; 1 lower left pressed 28/8/55; 1 right pressed 20/8/55.

FIG. 74: E. pseudokernerii, E. occidentalis and hybrids between them (about ¼ natural size).
plants are included in Fig. 74. Their parent was the plant of the F 1 pressed in October 1954. The F 2 seems to show rather more variation in habit than the F 1, the upper right-hand plant in Fig. 74 being noticeably like E. occidentalis.

The most strongly glandular plants of the hybrids of both generations were about as glandular as the plant of E. occidentalis grown in 1955, while others in the F 2 were more sparingly glandular, and one in each generation was eglandular. A plant of E. pseudokerner (E226), grown in 1954, had a very few glands and all were on one leaf.

Some impression of node of flowering and amount of branching can be gained from Fig. 74 and from Table 29, p.229. Only plants grown in the same year can be compared satisfactorily, because in 1955 all plants tended to flower at a later node than in 1954. The difference between E. pseudokerner and the F 2 hybrid in the number of the first flowering node in 1955 is statistically significant (P = .01).

Fig. 75, p.230, shows the flowers of this hybrid and its parents. Both the E. pseudokerner plants of 1953, for which the flowers are included, contributed to the hybrid seed sample. The flowers of the F 1 are intermediate between those of the parents in size and shape, except perhaps for those of the plant that came up in 1955. The flowers of this individual are so different as to suggest that the plant may have been a stray seedling. The flowers
TABLE 2Q: NODE OF FIRST FLOWER (F) AND NUMBER OF PRIMARY BRANCHING NODES (B) OF *EUPHRASIA* HYBRIDS AND PARENTS

<table>
<thead>
<tr>
<th></th>
<th><em>pseudokerneri</em></th>
<th><em>pseudokerneri x occidentalis</em></th>
<th><em>occidentalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of plants in 1954</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Mean of F</td>
<td>12</td>
<td><strong>9.3</strong></td>
<td>-</td>
</tr>
<tr>
<td>Range of F</td>
<td>-</td>
<td>8 - 10</td>
<td>-</td>
</tr>
<tr>
<td>Mean of B</td>
<td>8</td>
<td><strong>4.7</strong></td>
<td>-</td>
</tr>
<tr>
<td>Range of B</td>
<td>-</td>
<td>4 - 5</td>
<td>-</td>
</tr>
<tr>
<td><strong>No. of plants in 1955</strong></td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Mean of F</td>
<td>16.8</td>
<td>17</td>
<td>12.6</td>
</tr>
<tr>
<td>Range of F</td>
<td><strong>14 - 18</strong></td>
<td>-</td>
<td>9 - 17</td>
</tr>
<tr>
<td>Mean of B</td>
<td>9.8</td>
<td><strong>4</strong></td>
<td>4.3</td>
</tr>
<tr>
<td>Range of B</td>
<td>7 - 12</td>
<td>-</td>
<td>2 - 6</td>
</tr>
</tbody>
</table>

of the F 2 plants seem to be more variable than those of the F 1.

*E. salisburgensis var. hibernica x E. occidentalis* *(E230 x E192)*:

The parents of this cross are in two different subsections of the genus, namely, Angustifoliae (*E. salisburgensis*) and Ciliatae (*E. occidentalis*); they differ in habit about as much as *E. pseudokerneri* and *E. occidenta*-
E. PSEUDOKERNERI (E226)

1954

E. PSEUDOKERNERI × OCCIDENTALIS F1 (E226 × E192)

1955

E. PSEUDOKERNERI × OCCIDENTALIS F2 (E226 × E192)

1953 1954

E. OCCIDENTALIS (E192)

1954

E. SALISBURGENSIS VAR. HIBERNICA × OCCIDENTALIS (E230 × E192)

1953 1954 1955

E. SALISBURGENSIS VAR. HIBERNICA (E230)

FIG. 75 FLOWERS OF HYBRIDS AND THEIR PARENTS
FLOWERS OF EACH PLANT BRACKETED TOGETHER
lis in the cross just described, but there is in addition a
great difference in leaf shape. Four hybrid plants were
raised in 1954, and two are shown in Fig. 76. This shows
that they differ from *E. salisburgensis* var. *hibernica* in
their greater size, stouter stems and branches, less
flexuous branches and coarser foliage. The hybrids were
also more vigorous than either parent, but their seed-

**Top:** *E. salisburgensis* var. *hibernica* (E230)
2 plants: pressed 27/8/54.
**Bottom row:** *E. occidentalis* (E192)
3 plants: 1 left pressed 1/9/55;
1 centre pressed 2/9/53; 1 right
(grown on *Anthyllis vulneraria*)
pressed 24/9/53.

**E. salisburgensis var. hibernica x E. occidentalis** (E230 x E192)
2 plants: pressed 10/9/54.

**FIG. 76:** *Euphrasia* hybrid and its parents (about \( \frac{1}{2} \) natural size).
production was very poor, and this may have contributed to their vigour. The hybrids were as freely branched as *E. salisburgensis* var. *hibernica*, in spite of the very few and short branches of *E. occidentalis*.

The leaf shape of parents and hybrids can be seen in Fig. 77. The leaves of the hybrid are approximately intermediate, and the *E. salisburgensis* character remains very conspicuous.

Short-stalked glands were found with difficulty near the tips of the undersides of the leaves in all four hybrid plants; there were also scattered bristles which were quite easily seen. No glands could be found on the three plants of *E. salisburgensis* var. *hibernica* (E230) grown in 1954; the leaves had a few bristles, but these were shorter than those of the hybrid in at least one *E. salisburgensis*
plant. *E. occidentalis* (El92) was fairly freely glandular and bristly.

The flowers are shown in Fig. 75, p.230. The flowers of the hybrid are larger than those of both parents, and intermediate between them in shape; for instance, in the hybrid the dilation of the midlobe is similar to that in *E. salisburensis*, while the divergence of the lobes is similar to that in *E. occidentalis*.

**E. salisburensis x E. nemorosa (E515 x E608):**

*E. salisburensis* from a continental source was crossed with *E. nemorosa* in 1956. Seven plants of the hybrid were raised, one of which died about the time it began to flower. Herbarium specimens are shown in Fig. 78, p.234. There was only one plant of *E. salisburensis*, and it was pressed late so that seed could ripen. It differs considerably from var. *hibernica*. One plant of *E. nemorosa* and two plants of the hybrid are also shown; the hybrid plant on the left seemed to become established late, with the result that it is hardly branched at all; the one on the right became established earlier and produced long branches at one node. The hybrids look rather similar to *E. salisburensis*.

Leaf silhouettes of parents and hybrid may be compared in Fig. 79, p.235.

The flowers of *E. salisburensis* were only slightly different in size from those of *E. nemorosa* but distinctly different in shape; those of the hybrids were of similar
Left: E. anglica (E616)
2 plants: upper pressed 18/9/56; lower pressed 5/9/56.
Centre: E. anglica x E. hirtella
(E616 x E559)
1 plant: pressed 1/10/57.
Right: E. hirtella
(E559)
2 plants: 1 left pressed 30/8/56; 1 right pressed 13/8/56.

Left: E. nemorosa
(E608)
1 plant: pressed 11/10/56.
Centre:
E. salisburgensis
x E. nemorosa
(E515 x E608)
2 plants: 1 left pressed 20/9/57; 1 right pressed 14/10/57.
Right:
E. salisburgensis
(E515)
1 plant: pressed 15/10/56.

FIG. 78: Two Euphrasia hybrids and their parents (about \( \frac{1}{4} \) natural size).

size and intermediate in shape.

In 1958, one plant of the reciprocal cross, E. nemorosa x E. salisburgensis (E608 x E515), was raised; it was morphologically similar to the hybrids just described.
FIG. 79: Leaves of Euphrasia hybrids and their parents. 

The next hybrid to be described is the only one made so far between two different diploid species, the other crosses between diploids having been intraspecific.

**E. anglica** x **E. hirtella**, (E616 x E559):

These two species are very closely related. The most important difference between *E. anglica* and the form of *E. hirtella* used was the lack of branching in the latter, which can be seen in Fig. 78, p.234. Such branches as there were in *E. hirtella* appeared about a month later than those of *E. anglica*. The species also differed in the longer retention of the lower leaves of *E. hirtella*. Some seeds of the hybrid germinated in 1957, but only one plant grew well. In branching, it was intermediate between the parents, producing a single pair of well-developed branches which began growing at an early date. In leaf shape, the hybrid was also intermediate between the parents, although the latter differed little in this respect. In addition, the flower shape of the hybrid was intermediate, but the flower size was greater than that of the parents.
Hybrids of *E. micrantha* (E185A):

The occurrence of two spontaneous hybrids of *E. micrantha* (E185A) was described on p.189. Herbarium specimens of the hybrids are shown in Fig. 80, together with specimens of two of the parent populations.

**Top row:** Left - *E. micrantha* x ? (E185A x ?)
1 plant: pressed 1/9/54.
Right - *E. micrantha* (E185A)
1 plant: pressed 24/9/56.

**Bottom row:** Left - *E. confusa* (E183C)
1 plant (grown on *Anthyllis vulneraria*): pressed 3/9/53.
Right - *E. micrantha* x *E. confusa* (E185A x E183C)
1 plant: pressed 8/9/54.

**FIG. 80:** Hybrids of *E. micrantha* (E185A) (about 1/4 natural size).

As has been said before, the identity of the pollen parent of *E. micrantha* x *E. confusa* was indicated by the
yellow colour of the corolla. The intensity of this colour was as great as in *E. confusa*, and it therefore showed dominance in inheritance. This hybrid was sparingly branched, but it resembled *E. confusa* in its weak stem. The pollen parent of the other hybrid may have been *E. pseudokernerii*, or possibly a robust form of *E. confusa* from Dovedale.

The flowers of these hybrids, and of *E. micrantha*, may be seen in Fig. 81, p.238. Comparison of the flowers of the unidentified hybrid with those of *E. pseudokernerii* (E42 and E70) in Fig. 73, p.221, strongly suggests that *E. pseudokernerii* was in fact the pollen parent.

**Hybrids of *E. nemorosa* (E51A):**

All the Euphrasies to be described were grown with *Plantago lanceolata* as host-plant.

The origin of these hybrids from an outdoor trial in 1953 has already been explained (pp.186-7). A full account of the outdoor trial, with photographs of the living plants and with leaf and flower shapes, is given later and shows the morphology of the possible pollen parents, which were five other forms of *E. nemorosa* and one of *E. stricta* or hybrids of it with *E. nemorosa*. The ovule parent, however, is described here also. This form (E51A) of *E. nemorosa* (Fig. 82, p.239) was dwarf and flowered relatively early. The leaves were thick, pale green, dull, and flat between the veins. The bracts were very broad and few-toothed. A hybrid plant shown in Fig. 83, p.240, resembled the pollen
E. *MICRANThA* (E185A)

E. *MICRANThA* (E185A) ACCIDENTALLY CROSSED (POLLEN PARENT UNCERTAIN)

E. *MICRANThA* (E185A) ACCIDENTALLY CROSSED WITH E. *CONFUSA* (E183C)

HYBRIDS 1954

HYBRIDS 1954

MATERNAL TYPE 1954

HYBRIDS 1955

MATERNAL TYPE 1954

E. *NEMOROSA*: OFFSPRING OF E151A

FIG. 81 FLOWERS OF HYBRIDS PRODUCED ACCIDENTALLY IN CULTIVATION. FLOWERS OF EACH PLANT BRACKETED TOGETHER
FIG. 82: Plant of *E. nemorosa* (E151A) at Leicester, 29 July 1953.
Fig. 83: Plant of a hybrid of E. nemorosa (EL14) at Cambridge, 3 September 1954.
parents more than the maternal type in habit, but something of the leaf-shape of the female parent can be detected in it.

In 1954, seed of *E. nemorosa* (El51A) from the 1953 outdoor trial was sown, and 79 Euphrasia seedlings were planted out in the garden. An arbitrary selection of the surviving plants was preserved, and leaves and flowers were mounted. Leaf specimens are shown in Fig. 84, those of the hybrid and maternal types being arranged separately. These

![Leaf specimens](image)

**FIG. 84**: Leaves of offspring of *E. nemorosa* (El51A). Where there are two leaves from one plant they are linked by underlining.
mounted leaves were selected for maximum number of teeth, and were taken from the 4th to 13th nodes after branching ceased. Some more seeds germinated in 1955, and a few plants were raised. Leaves of these plants were selected for mounting from about the 12th node, counting from the base of the plant. In plants of the maternal type, the number of leaf teeth did not exceed four in 1954 and three at about the 12th node in 1955, and the teeth were larger than the more numerous teeth of the hybrids.

In 1953 the population EL51A had very uniform and distinctive flowers, in which the lobes of the lower lip were long, narrow, and shallowly emarginate, and in which the midlobe was rather suddenly dilated near the tip. It is easy to distinguish the hybrid flowers in the progeny grown in 1954 and 1955 (Fig. 81, p. 238).

Conclusion.

From this account of the morphology of hybrid Eu-
phrasias it may be concluded that hybrids are usually intermediate between the parents, or exhibit combinations of characters of the parents, but that occasionally the hybrids are outside the range of both parents in certain characters. Where an F2 generation was obtained, it was found to be more variable than the F1.

The Cytology of Euphrasia Hybrids.

The cytology of a few of the hybrids just described was investigated by the method used previously (p. 144).
E. pseudokernerli x E. nemorosa (E42 x 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 was the interpretation quite clear. No irregularities were definitely 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. pseudokernerli x E. scotica (E42 x 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, most frequently two in number, but as many as six were counted (Fig. 85, p. 244). Very little was seen of other stages of meiosis, but in one Anaphase II a group definitely 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 re-forming nuclei, while in another more advanced cell there were no stray chromosomes.

E. salisburgensis var. hibernica x E. occidentalis (E230 x E192):

Notes were made on about 110 cells derived from two plants. Most of the preparations were good, and definite
FIG. 85: Pollen mother cell meiosis in *E. pseudokerneri* x *E. scotica* (E42 x E120). There are four univalents, one possible univalent or bivalent, and 19 definite bivalents. Interpretations of some cells were obtained. All stages of meiosis were seen. Many univalents were always present at Metaphase I; these were counted as accurately as possible, and the results for 40 cells were as follows:

<table>
<thead>
<tr>
<th>Univalents</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
</tr>
</tbody>
</table>

Where an odd number of univalents was visible, one was added to the count, on the assumption that they always occur in pairs. A photograph of this stage of meiosis is given in Fig. 86, p. 245. 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. 86), while others...
FIG. 86: Pollen mother cell meiosis in *E. salisburgensis* var. *hibernica* x *E. occidentalis* (E230 x E192).

Above - Metaphase I, showing 20 univalents and 12 bivalents. 
Below - Telophase I, showing division of univalents.

were to be seen in the peripheral region and probably did not divide. Finally, all chromosomes joined one or other of the Telophase nuclei.

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. 87). However, the laggards all eventually joined a nucleus (Fig. 87). Such an irregular meiosis can rarely lead to the production of a pollen grain with a normal set.

---

FIG. 87: Pollen mother cell meiosis in *E. salisburgansis* var. *hibernica* x *E. occidentalis* (E230 x E192).

*Above* - Anaphase II. Chromosomes that divided during the first division now lagging. *Below* - Telophase II. All chromosomes have joined, or nearly joined, nuclei.
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.

Production of Normal Pollen in Euphrasia 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 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 and at once wrote them down. They are given in Table 30, p. 248.

In the hybrid E. pseudokerneri × E. scotica pollen counts were first made from three plants; two of them produced about 70% of normal pollen, and the third produced
TABLE 10: PRODUCTION OF NORMAL POLLEN IN SPECIES AND HYBRIDS OF EUPHRASIA

<table>
<thead>
<tr>
<th>Euphrasia</th>
<th>Production of normal pollen (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents</td>
<td></td>
</tr>
<tr>
<td>pseudokerneri (E42, E70) &quot; (E226)</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>occidentalis (E192)</td>
</tr>
<tr>
<td></td>
<td>salisburgensis var. hibernica (E230)</td>
</tr>
<tr>
<td>Cross within one species</td>
<td></td>
</tr>
<tr>
<td>anglica x anglica (E76 x E168)</td>
<td>100%</td>
</tr>
<tr>
<td>Crosses within one Series</td>
<td></td>
</tr>
<tr>
<td>pseudokerneri x nemorosa (E42 x E154) &quot;</td>
<td>? 100%</td>
</tr>
<tr>
<td></td>
<td>x occidentalis (E226 x E192)</td>
</tr>
<tr>
<td>Crosses between Series</td>
<td></td>
</tr>
<tr>
<td>pseudokerneri x brevipila (E70 x E81) &quot;</td>
<td>85%</td>
</tr>
<tr>
<td></td>
<td>x scotica (E42 x E120)</td>
</tr>
<tr>
<td>brevipila x scotica (E81 x E120)</td>
<td>85%</td>
</tr>
<tr>
<td>micrantha x confusa (E185A x E183C)</td>
<td>85%</td>
</tr>
<tr>
<td>Cross between Subsections</td>
<td></td>
</tr>
<tr>
<td>salisburgensis var. hibernica x occidentalis (E230 x E192)</td>
<td>17-24%</td>
</tr>
</tbody>
</table>

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 30 shows a fairly good correspondence between pollen fertility and the affinity of the hybrids' parents although, in view of what has been said, such a conclusion must be accepted tentatively.

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.

Seed-Production and Germination in Euphrasia Hybrids.

The seed-production resulting from artificial self-pollination of some of the hybrids is given in Table 31. The large bad seeds were not less than half the length of

<table>
<thead>
<tr>
<th>TABLE 31: PRODUCTION OF SEED BY EUPHRASIA HYBRIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphrasia</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Crosses within one Series</td>
</tr>
<tr>
<td>pseudokerneri x occidentalis (E226 x E192)</td>
</tr>
<tr>
<td>anglica x hirtella (E616 x E559)</td>
</tr>
<tr>
<td>Crosses between Series</td>
</tr>
<tr>
<td>pseudokerneri x brevipila (E70 x E81)</td>
</tr>
<tr>
<td>&quot; x scotica (E42 x E120)</td>
</tr>
<tr>
<td>micrantha x confusa (E185A x E183C)</td>
</tr>
<tr>
<td>Cross between Subsections</td>
</tr>
<tr>
<td>salisburgensis var. hibernica x occidentalis (E230 x E192)</td>
</tr>
<tr>
<td>salisburgensis var. hibernica x occidentalis (E230 x E192) (open-pollinated)</td>
</tr>
</tbody>
</table>

Note: in a few capsules some additional seeds were lost.
normal seeds, and were usually empty but occasionally partly filled. In *E. salisburgensis* var. *hibernica* x *E. occidentalis* seed production was in fact poorer than it appears to have been from Table 31, because in addition to the five self-pollinated capsules mentioned there were eleven undeveloped self-pollinated capsules, and interspersed with the eight open-pollinated capsules mentioned there were five undeveloped ones. The other hybrids produced, as a result of self-pollination, only fertile capsules, except for *E. anglica* x *E. hirtella* which produced a single undeveloped one not included in the Table.

The hybrid *E. salisburgensis* x *E. nemorosa* (E515 x E608) is not included in Table 31 because the minute empty seeds that were produced were not always counted; but, of 32 self-pollinated capsules,

16 failed to develop,
13 developed partially and dehisced but contained only minute empty seeds,
1 was fairly well-developed, with 1 large empty and several small empty seeds, and
2 were partly or fully developed, each with 1 good seed and some small empty ones;

and, with open pollination, the 12 capsules that showed some enlargement gave 10 good seeds, over 70 small empty seeds, 9 large empty seeds and 3 partly-filled ones.

Large bad seeds are rarely produced by the self-pollination of non-hybrid plants (p.198); their production by the self-pollination of *E. pseudokerneri* x *E. brevipila*, *E. pseudokerneri* x *E. occidentalis*, *E. micrantha* x *E. confusa*
and \textit{E. anglica} x \textit{E. hirtella} may therefore be an indication of hybrid sterility affecting the production of good seed. In \textit{E. pseudokernerli} x \textit{E. scotica} the large proportions of both types of bad seed are doubtless due to hybrid sterility, while \textit{E. salisburgensis} var. \textit{hibernica} x \textit{E. occidentalis} and \textit{E. salisburgensis} x \textit{E. nemorosa} were almost completely sterile. The seed-production of \textit{E. anglica} x \textit{E. hirtella} was unexpectedly poor, in view of the close similarity between these two species.

The germination of the hybrid seed is given in Table 32. It will be seen that germination occurred in two hybrids, the one with the morphologically most similar parents, and that with the morphologically least similar parents. Of the former, \textit{E. pseudokernerli} x \textit{E. occidentalis},

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|}
\hline
\textbf{Euphrasia} & \textbf{No. of seeds germinating} & \textbf{Number sown 1955 1956} \\
\hline
\textbf{Cross within one Series} & & \\
pseudokernerli x occidentalis (E226 x E192) & 65 & 13 1 \\
\hline
\textbf{Crosses between Series} & & \\
pseudokernerli x brevipila (E70 x E81) & 85 & 0 0 \\
x scotica (E42 x E120) & 88 & 0 0 \\
micrantha x confusa (E185A x E183C) & 200 & 0 0 \\
\hline
\textbf{Cross between Subsections} & & \\
salisburgensis var. hibernica x occidentalis (E230 x E192) & 8 & 0 0 \\
salisburgensis var. hibernica x occidentalis (E230 x E192) (open-pollinated) & 18 & 5 0 \\
\hline
\end{tabular}
\caption{Germination of F2 seed of \textit{Euphrasia}}
\end{table}
seven plants matured in 1955 and one in 1956, and these have been described on pp. 226-229; the remaining seedlings of this hybrid died. Seven seedlings had come up by 21 March 1955, and five more by 28 March of which three were very pale yellow. On 4 April another seedling had come up, but two of the three pallid ones had disappeared and only the rotting remains of the cotyledons and hypocotyl of the other were still visible. Presumably these three seedlings had been unable to survive owing to lack of chlorophyll. One other seedling died before 16 April, on which date the 9 survivors were potted up, and two of these had died by 17 May. The five seedlings of the other hybrid to germinate, *E. salisburgensis* var. *hibernica* x *E. occidentalis*, are accounted for as follows: by 16 April two had died and one appeared to be dying; the two healthy ones were potted up on this date, when rather well-grown; one was dead by 27 April, but the other was quite vigorous; however, on 1 May it had been eaten back to the cotyledonary node by a slug and died shortly afterwards.

It is difficult to understand why the seed of all the other hybrids should have been inviable, but it might possibly have been that the storage conditions were unsuitable for the seed of some hybrids. Because of this possibility, some more hybridizations were performed in 1956, duplicating the earlier crosses as far as possible. The seed production for two of the 1956 crosses, *E. anglica* x *E. hirtella* and *E. salisburgensis* x *E. nemorosa*, has been
given already in Table 31, p. 249, and on p. 250 respectively. In *E. anglica* x *E. hirtella*, which was not a duplicate of an earlier cross, 5 out of 56 seeds of the hybrid germinated in 1958, all five seedlings being brought to maturity. In *E. salisburgensis* x *E. nemorosa* (a cross comparable to *E. salisburgensis* var. *hibernica* x *E. occidentalis* grown in 1954) 11 seeds produced by open-pollination of the hybrids gave rise to 6 seedlings, and two plants were raised in 1958 which were as sterile as the F1. Seed produced by the hybrids *E. pseudokerner* x *E. nemorosa* (E609 x E608) and *E. pseudokerner* x *E. brevipila* (E609 x E623) (a duplicate of an earlier cross) also germinated in 1958 and gave rise to mature plants. These results strongly suggest that the previous failure of hybrid seed to germinate was not due to hybrid sterility.

VI. Summary of Breeding Studies

A paper, already published, on the chromosome numbers of *Euphrasia* is supplemented by photographs of chromosome counts reported in it, and a few additional ones. A further example of a population with meiotic irregularities is described.

It is shown that self-pollination takes place at an early stage in the development of the smaller flowers, and that it takes place less readily, and at a later stage, in the larger flowers, which may be to some extent dependent
on insect-pollination for a full seed-set.

Pollen-eating and nectar-eating insects (Diptera and Hymenoptera) visit the flowers, and most of them appear to be capable of causing cross-pollination. Insect pollinators were responsible for the occurrence of a high proportion of hybrids in the progeny of some Euphrasias grown in a garden, and were presumably involved in the production of a few hybrids between plants grown in the greenhouse.

Hybrids occur frequently in nature, and the number of individuals in hybrid populations may be very large, suggesting that the hybrids are self-fertile or that the backcrosses are fertile. The number of plants in a hybrid population is probably ultimately determined by selection rather than by the affinity of the parents.

There is no sign of apomixis in the species of Euphrasia used in these breeding studies. The species give good seed-yields on enforced self-pollination, and some of the seed is viable, but there is no accurate comparison with the viability of wild seed.

When diploids are pollinated by tetraploids, the capsules develop normally, and normal numbers of seeds are produced; but, although the testas are of normal dimensions, the seeds are empty. In the reverse type of cross, capsule and seeds do not usually develop at all, but undersized seeds with some contents are occasionally produced. None of these undersized seeds germinated.

When species alike in chromosome number are crossed,
the seed-production is **practically** normal, even when species of Subsection Angustifoliae are crossed with species of Subsection Ciliatae. The hybrid seed germinates well and gives rise to plants intermediate between the parents in most characters, but more like one parent than another in a few characters; rarely, hybrids are outside the range of both parents in some character. Intraspecific hybrids give similar results.

The hybrids between the species most closely related according to Pugsley's classification showed full pollen-fertility and normal production of good seed. Meiosis in one hybrid of this type was investigated and appeared normal. Intraspecific crosses also showed normal seed-production. Somewhat less closely related parents gave hybrids with distinctly reduced proportions of good pollen and seed; one hybrid was investigated cytologically, and it showed frequent pairing failure at meiosis. The hybrids of the least closely related parents produced very little good pollen and seed, and one hybrid showed very irregular meiosis. The F2 seed of two hybrids grown in 1954 germinated; one hybrid had distantly related, and the other closely related, parents. The seedlings of the former died; of the latter, three albino seedlings died but several others grew to maturity. Seed of four hybrids grown in 1957 germinated in 1958.

There is, therefore, a **fairly** good correspondence between the morphological affinity of the parents of a
hybrid (as indicated by Pugsley's classification) and the fertility of the hybrid. The 1957-58 work seems to indicate that most hybrids can produce an F2 generation, but even the closest interspecific crosses show slight signs of sterility of the F1.
I. Comparison of Seven Euphrasia Samples grown in the Garden.

Description of Experiment.

The "whalehide pot" method of cultivation was the one used in this experiment, which was carried out at Leicester in 1953 as described on pp.130-133. Each pot contained one seedling of Euphrasia and one of Plantago lanceolata. The taxonomy of the Euphrasias grown will be discussed later. At the time of collecting in 1952, all the seven samples were thought to be E. nemorosa, and they included some that resembled each other closely and some that were apparently distinct from one another. The aim of the experiment was to compare the offspring of these wild populations when grown in the same environment. Six of the samples were grown in a set of randomized blocks, an arrangement which permits an analysis of variance to be made on the statistical data. There were six blocks, each consisting of six rows of 14 Euphrasia plants each. Each sample of Euphrasia was represented by one row in each block. The individual plants were assigned to the rows at random, and the order of the rows in each block was also determined at random. The seventh sample, of which few seedlings were available, was grown in a row of ten plants near the main experiment.

On four occasions from mid-May to mid-June a record was made of the survival of the Euphrasias, signs of establishment on the host, and signs of disorder in the
plants. These records have been reported already in the account of cultivation (pp. 130-133).

Leaves and flowers were taken from the plants and mounted on cellulose tape on glass, as described previously (p. 210). From each plant two leaves were taken, one subtending the last-but-one normally-developed branch or pair of branches (to be referred to as 'leaf 2') and one from the fourth node above the uppermost branch or pair of branches (to be referred to as 'bract 4'). One flower from each plant was mounted; the aim in collecting these was to obtain mature full-sized flowers which had not begun to wrinkle or shrink. Most of the flowers chosen were therefore ones which had not been shed but which could be pulled off easily. The mounting was begun on 29 July 1953.

Finally, all the plants were pressed between 27 August and 7 September, except for some which were pressed earlier because they were wilting.

The height of the pressed plants was measured, and various measurements were made on the mounted leaves and flowers. For this purpose, the lantern-plate cover-glasses on which they were mounted were put in a projector and the measurements were made on their enlarged images. In addition, photographs were taken of individual plants in the garden, and photographic contact-prints were made of the mounted leaves and flowers.

The *Euphrasia* seed for this experiment was extracted from many different plants of large or fairly large gather-
ings of herbarium specimens. The samples will be referred to by their serial numbers. The six samples used in the main part of this experiment were:

- **E151A** Juniper Top, Box Hill, Surrey; flinty, rabbit-grazed turf on chalk;
- **E166** Watlington Hill, Oxon.; chalky field;
- **E167A** near Medmenham, Bucks.; chalky field (with E167B, see below);
- **E210** Waltham Quarry, Waltham on the Wolds, Leics.; grassland on oolite;
- **E211** near Croxton Kerrial, on Leics.-Lincs. border; grassy track on oolite, about 6 miles from E210;
- **E215** Bedford Purlieus, Northants; woodland ride on oolite, about 19 miles from E211 and about 17 miles from E210.

The additional group of 10 plants grown nearby, numbered E167B, was from the same locality as E167A and was growing mixed with it; E167A and E167B were separated by differences in leaf-shape and flower-shape. At Box Hill the population of E151A was in contact with and apparently hybridizing with *E. pseudokerneri*. Plants thought to be hybrids were excluded from the gathering.

**Effect of Replacement of Dead Seedlings.**

Many seedlings of *Euphrasia*, and some of the host, were replaced from 22 to 26 April, about four weeks after the initial potting-up and early replacement of the Euphrasias and hosts (p.130). Survival was slightly less good where replacements had been made, but the percentage of survivors probably established on the host was approximately the same.
whether replacements had taken place or not.

Variation Between Populations in Survival and Establishment.

Table 33 shows that there was considerable variation in survival up to 31 May between the different samples. This variation was greater than that between the blocks, each of which contained all the samples. The table shows a similar result for establishment on the host.

**TABLE 33: SURVIVAL AND ESTABLISHMENT OF EUPHRASIA IN 1953 IN THE GARDEN**

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of Euphrasias alive on 22-26 April that survived until 31 May</th>
<th>% of surviving Euphrasias probably established on host on 31 May</th>
</tr>
</thead>
<tbody>
<tr>
<td>E151A</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>E166</td>
<td>66</td>
<td>83</td>
</tr>
<tr>
<td>E167A</td>
<td>81</td>
<td>84</td>
</tr>
<tr>
<td>E210</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td>E211</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>E215</td>
<td>82</td>
<td>67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Block</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>87</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>81</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>81</td>
</tr>
</tbody>
</table>
Deaths of plants later in the season, after they had become established on the host, were mostly due to the fraying of the stem base (pp.131-133). Many plants which did not die wilted readily in dry weather from this cause. However, the sample El51A, which suffered the heaviest mortality before establishment, showed no ill effects from stem-fraying, apparently because of its dwarf habit.

**Variation Between Populations in Habit.**

Variation in habit is illustrated by photographs of some of the living plants, and by height measurements. Those of the leaf characters which cannot be seen in the silhouettes given later will also be considered here.

All samples were much more bushy than their wild parents had been. Sample El51A (Fig. 82, p.239) was dwarf, with short internodes, few branches, and a relatively low node of flowering. The leaves were very thick and developed anthocyanin readily; their green colour was pale and they were not shiny. The areas between the veins were flat on the upper surface of the leaves and very slightly concave beneath; the veins appeared on the upper surface as narrow grooves. In all these characters El51A was different from all the other samples, except El67A.

Sample El66 (Fig. 88, p.262) was characterized in habit by its small leaves which left the branches more exposed than in other samples.

The remaining samples were all very similar in habit,
FIG. 88: Plant of El66, 2 August 1953.
but El67A (Fig. 89, p. 264) was distinguishable by its leaf shape, which will be described later, and by the considerable development of anthocyanin in its leaves. Of the characters under consideration in this section, this anthocyanin production was the only one in which El67A resembled El51A.

The other three samples, E210, E211 and E215, could not be distinguished from one another in the garden by habit and foliage. Plants of E211 and E215 are illustrated in Fig. 90, p. 265, and Fig. 91, p. 266, respectively. It will be noted that the leaf surface was similar to that of El66 (Fig. 88, p. 262).

The measurements of plant height, together with numerical data obtained for ten other characters, (Table 34, p. 267) have been subjected to an analysis of variance. This work was kindly carried out by Mr. D. A. Wilkins of the Scottish Plant Breeding Station. It was found that, in all eleven characters, differences existed among the populations significant at the 0.1% level of probability.

For the character of plant height (character 1), Table 35, p. 268, shows that El51A was significantly shorter than all other samples, and that sample El67A was significantly shorter than the two tallest samples. Table 34 shows that El51A was in fact only about half the height of E210 and E211.
FIG. 90: Plant of E211, 3 August 1953.
FIG. 91: Plant of B215, 28 August 1953, photographed at an advanced stage.
TABLE 34: AVERAGES OF MEASUREMENTS MADE ON EUPHRASIAS

(Unit of measurement = $\frac{1}{7}$ mm., unless otherwise stated; greatest and least averages for each character are underlined)

<table>
<thead>
<tr>
<th>Population</th>
<th>E151A</th>
<th>E1665167A</th>
<th>E210</th>
<th>E211</th>
<th>E215</th>
</tr>
</thead>
</table>

**Habit character**

1. Height of plant (in cm.) after pressing.  
   12.7 22.7 18.9 25.4 25.0 22.6

**Foliar characters**

2. Length of *bract 4* (see p.258).  
   65.9 71.7 92.7 85.2 83.4 75.2

3. Breadth of *bract 4*  
   78.2 72.8 83.0 90.8 84.2 81.8

4. Greatest no. of teeth on a side of *bract 4*.  
   3.72 6.35 5.08 7.70 6.80 7.13

5. Length of distal side of a tooth on the widest part of *bract 4*.  
   13.2 10.9 14.6 13.8 12.2 13.4

**Floral characters**

   51.2 46.0 54.5 53.0 53.2 55.5

7. Length of lower lip of corolla plus tube.  
   57.3 52.5 63.3 61.2 60.7 64.2

8. Length of mid-lobe of lower lip of corolla.  
   20.3 14.9 19.3 18.9 18.7 19.0

   4.1 4.2 5.7 5.5 6.3 6.9

   9.4 8.5 10.3 12.0 11.2 11.0

   15.9 13.8 16.6 19.0 19.1 21.5
TABLE 35: CHARACTERS IN WHICH PAIRS OF EUPHRASIA SAMPLES
SHOWED A STATISTICALLY SIGNIFICANT DIFFERENCE (P = 0.1%)  
(Characters numbered as in Table 34, p.267)

<table>
<thead>
<tr>
<th>Pairs of samples</th>
<th>Characters in which pairs of samples differed significantly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Habit</td>
</tr>
<tr>
<td>E151A and E166</td>
<td>1</td>
</tr>
<tr>
<td>&quot;    &quot; E167A</td>
<td>1</td>
</tr>
<tr>
<td>&quot;    &quot; E210</td>
<td>1</td>
</tr>
<tr>
<td>&quot;    &quot; E211</td>
<td>1</td>
</tr>
<tr>
<td>&quot;    &quot; E215</td>
<td>1</td>
</tr>
<tr>
<td>E166 &quot; E167A</td>
<td>2, 4, 5</td>
</tr>
<tr>
<td>&quot;    &quot; E210</td>
<td>3, 4, 5</td>
</tr>
<tr>
<td>&quot;    &quot; E211</td>
<td>5</td>
</tr>
<tr>
<td>&quot;    &quot; E215</td>
<td>4</td>
</tr>
<tr>
<td>E167A &quot; E210</td>
<td>1</td>
</tr>
<tr>
<td>&quot;    &quot; E211</td>
<td>2, 4</td>
</tr>
<tr>
<td>E210 &quot; E211</td>
<td>9</td>
</tr>
<tr>
<td>&quot;    &quot; E215</td>
<td>9, 11</td>
</tr>
<tr>
<td>E211 &quot; E215</td>
<td>7, 11</td>
</tr>
</tbody>
</table>

Variation Between Populations in Leaves.

The leaves of the populations are illustrated in Figs. 92 and 93, pp.269 & 270. Differences between samples E166, E210, E211 and E215 are not very noticeable in the silhouettes, but E151A and E167A are conspicuously distinct.
FIG. 92: Leaves of Euphrasia plants grown in the garden, 1953. In each sample the leaves represent all the surviving healthy plants in a single row of the experimental layout.
FIG. 93: Leaves of *Euphrasia* plants grown in the garden, 1953. In each sample the leaves represent all the surviving healthy plants in a single row of the experimental layout.
Table 35, p.268, shows which of the differences are statistically significant. It is noteworthy that there are no significant foliar or habit differences between E210, E211 and E215, which were the samples that could not be distinguished by these characters when they were being grown. A comparison of the figures for the length and breadth of the leaves (Table 34, p.267) indicates a variation between the samples in leaf shape.

For two samples, Figs. 92 and 93 also include 'leaf 2' (p.258) taken from the same plants as 'bract 4'. 'Leaf 2' has fewer, shorter and less acute teeth than 'bract 4', and it is also extremely large, indicating the luxuriance of the plants.

Leaves of two untypical plants, plant 1 of El51A and plant 2 of El67A, have been included in Fig. 92. These will be discussed later.

Variation Between Populations in Flowers.

This variation is also shown by Tables 34 and 35, and some flowers are illustrated in Fig. 94, p.272. It is clear that samples El51A and El66 were the most distinctive in flower shape. E210, E211 and E215, with no significant habit or foliar differences, are found to differ in their flowers. The tables show that E210 has a less deeply emarginate midlobe than the other two, E215 has a longer lower lip and tube than E211, and E215 has a wider (more dilated) midlobe than both E210 and E211.
FIG. 94: Flowers of *Euphrasia* plants grown in the garden, 1953.
Variation Between Populations in Number of Leaf Teeth.

The number of teeth on a side of 'bract 4' attained for each population the following maxima:

<table>
<thead>
<tr>
<th>Population</th>
<th>Teeth</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>E151A</td>
<td>4</td>
<td>3/4</td>
</tr>
<tr>
<td>E166</td>
<td>7</td>
<td>1/2</td>
</tr>
<tr>
<td>E167A</td>
<td>6</td>
<td>1/8</td>
</tr>
<tr>
<td>E210</td>
<td>9</td>
<td>1/6</td>
</tr>
<tr>
<td>E211</td>
<td>8</td>
<td>1/6</td>
</tr>
<tr>
<td>E215</td>
<td>8</td>
<td>1/6</td>
</tr>
</tbody>
</table>

When the two untypical plants (p.271) were omitted, each population produced its maximum tooth number in each of the six blocks of the field trial. It seems, therefore, that there was a characteristic maximum tooth number for each population, which was not attained by all individuals. It is interesting to note that E210 had a greater maximum tooth number than E211 and E215, which it closely resembled (p.271).

Variation Within Populations.

Mr. Wilkins calculated the standard deviations of each of the six populations for one foliar and one floral character. These are given in Table 36, p.274; they show that E210 and E211 were the most variable populations. The wild populations at the localities of E210 and E211 were numerically larger than the other four populations, and they were certainly sampled over a larger area than the others, except possibly E167A, which also formed an
### TABLE 36: STANDARD DEVIATIONS FOR EUPHRASIA POPULATIONS

(Unit of measurement = \( \frac{1}{2} \) mm.)

<table>
<thead>
<tr>
<th>Population</th>
<th>Standard deviation for length of 'bract 4'</th>
<th>Standard deviation for greatest width of midlobe of lower corolla-lip</th>
</tr>
</thead>
<tbody>
<tr>
<td>E151A</td>
<td>6.79</td>
<td>1.52</td>
</tr>
<tr>
<td>E166</td>
<td>12.80</td>
<td>1.27</td>
</tr>
<tr>
<td>E167A</td>
<td>11.37</td>
<td>2.62</td>
</tr>
<tr>
<td>E210</td>
<td>16.40</td>
<td>2.73</td>
</tr>
<tr>
<td>E211</td>
<td>13.70</td>
<td>3.51</td>
</tr>
<tr>
<td>E215</td>
<td>11.32</td>
<td>2.28</td>
</tr>
</tbody>
</table>

extensive population although it was not extremely abundant.

Population E151A was very uniform. However, it included one untypical plant (p.271) that stood out sharply from the rest. Its flowers are shown in Fig. 95, and a photograph of the plant is given in Fig. 96, p.275.

Compared with the other plants of this population, it will be seen from Fig. 92, p.269, that this plant had 5 teeth on a side of 'bract 4' instead of 3 or 4; from Fig. 94, p.272, and Fig. 95 that the corolla lobes were broader; and from

FIG. 95: Left - Flowers of untypical plant of E151A grown in 1953. Right - A leaf from the 11th node and two flowers of a similar plant (E469B) found wild at the locality of E151A in 1954.
Fig. 82, p.239, and Fig. 96, p.275, that the leaf surface was not so flat, the habit more branched, and the flowers more abundant.

This untypical plant was evidently a hybrid between E151A and another species and had a different locality, and it

Fig. 96: Untypical specimen of E151A, 2 August 1953.
Fig. 82, p.239, and Fig. 96, p.275, that the leaf surface was not so flat, the habit more branched, and the flowers more abundant.

This untypical plant was evidently a hybrid between EI51A and E. pseudokernerii which grew at the same locality, and it seems likely that it was an F1 hybrid, because hybrid plants were carefully excluded in collecting EI51A. When the locality of EI51A was revisited in 1954, plants considered to be hybrids of this form of E. nemorosa with E. pseudokernerii were again found. One of them was similar in flower-shape and leaf-shape to the cultivated hybrid plant just described, and it may also have been an F1 hybrid (Fig. 95, p.274).

The other plant with an untypical leaf shape (p.271) belonged to population EI67A. The leaf of this plant was similar in shape to those of the five other populations, but it was unlike EI51A in tooth-number; it was the only 'bract 4' leaf of EI67A with more than six teeth on one side. Leaves of this shape also occurred in EI67B, of which ten plants had been planted near the six other populations; the seven surviving plants of EI67B are represented in Fig. 97, p.277. It will be seen from these photographs that the leaves of plants 6 and 10 resembled those of EI67A, while the leaves of plants 1, 2, 4, 5 and possibly 8 resembled those of the one untypical specimen of EI67A. It appears that two types of Euphrasia occurred at the locality of EI67, and that the groups A and B into which
FIG. 97: Leaves, bracts and flowers of population El67B.
they were sorted were not uniform in leaf-shape, although in cultivation one type of leaf predominated heavily in El67A, and the other predominated slightly in El67B. It seems probable that the wild population, El67, consisted of two forms, distinguished chiefly by leaf-shape. In El67B, plant 6 (Fig. 97, p.277) was perhaps intermediate between the two forms in leaf-shape. Cultivated plants intermediate in leaf-shape also occurred in El67A, and four of these (plants 3, 7, 12 and 14) are illustrated in Fig. 98. This variation in leaf-shape in both El67A and El67B suggests that the two forms were hybridizing.

FIG. 98: 'Bract 4' of plants of El67A from a different row from that illustrated in Fig. 92, p.269.
Comparison of Wild and Cultivated Plants.

The wild plants of EI51A were extremely dwarf and compact, but freely branched. The leaves were few-toothed, and fleshy-looking but shiny. The plants were seeding freely when collected on 24 July 1952, which is an early date for *E. nemorosa* to be in such an advanced condition. EI66 was taller and had longer internodes than EI51A, but was dwarfer and had smaller leaves and flowers than the other populations. EI67A had a distinctive leaf-shape, but was otherwise rather similar in habit to the remaining three samples. These three were all collected on the same day, and appeared to differ slightly from one another, E211 having stout dense flowering spikes, and E215 particularly long internodes, compared with E210.

Population EI51A, which was the most distinct in the field, was also the most distinct in cultivation. Its few leaf teeth, dwarf habit, and early flowering were shown to be hereditary, although the internodes were not quite as short in cultivation as in nature. The leaves became even more fleshy in cultivation, and developed a flat, non-shiny surface.

In cultivation, population EI66 was little different from E210, E211 and E215, except in its small leaves and flowers; it looked about as distinct as it did in the wild.

EI67A in the main retained its characteristic leaf-shape in cultivation.

The slight differences which E210, E211 and E215
showed in the wild disappeared in cultivation, where they could not easily be distinguished by eye.

The conditions of cultivation, which allowed the plants to develop with maximum vigour, probably helped to bring out such differences as there were between the populations in leaf-shape, number of leaf-teeth and in the texture of the leaves. The experiment gave indications of the type of heritable variation to be found between wild populations. Thus large differences were found in habit, leaf-shape, number of leaf-teeth, and flower size and shape. In addition, forms superficially alike showed small differences in number of leaf-teeth and shape of flower. Later experience in the field, and in cultivating smaller numbers of plants in the greenhouse, has shown that nearly all populations of a species differ visibly, and often quite conspicuously, from one another. Some of the differences visible between wild populations are likely to be retained in cultivation, but others not evident in the wild often become apparent in cultivated samples.

**Taxonomy.**

It was hoped that this garden experiment might throw some light on *E. nemorosa* var. *calcarca* Pugsl., as two samples, E151A and E166, were from chalk downs, and showed some of the characters given for this variety. E151A was distinct from all the other populations, and it seemed possible that it might be a recognizable taxon. To
investigate this, I spent two days in 1954 at Box Hill and Mickleham Downs. The area from which EI51A had been collected was revisited and the same form collected again. Several other gatherings of *E. nemorosa* from both grassy fields and dry chalky slopes were made, but none was at all like EI51A. They appeared to be fairly normal forms of *E. nemorosa*, although those from the poorest habitats were the most dwarf. The form represented by EI51A would deserve taxonomic recognition on morphological grounds, provided it could also be found in other localities. It is not identical with *E. nemorosa* var. *calcarea*, the type of which from Epsom, Surrey, appears to be a fairly normal form of *E. nemorosa*. Specimens of a better-marked form are illustrated under this varietal name in Pugsley's revision. These specimens came from Box Hill, and in 1951 I collected specimens identical with them from a point very near where I later found EI51A. In fact, these specimens of mine might have been EI51A showing a different appearance late in the year, for on visits earlier in the season in 1952 and 1954 I was unable to find this form. It is more likely, however, that these specimens were hybrids between EI51A and *E. pseudokerneri*. It seems possible that Pugsley had plants from this area of Box Hill chiefly in mind when describing *E. nemorosa* var. *calcarea*, but that he made an unfortunate choice of a type specimen. Although EI66 was similar to the description of *E. nemorosa* var. *calcarea*, my work in the field and in the herbarium does not suggest
that a distinct form of *E. nemorosa*, occurring repeatedly on chalk and limestone downs, can be recognized.

Regarding the plants from Medmenham (E167A & B), I consider that two species were present, *E. stricta*, represented by nearly all the cultivated plants of E167A, and *E. nemorosa*, represented by one cultivated plant of E167A and four of E167B. As already suggested, there were probably also hybrids among the wild plants and among those cultivated. Other samples of *E. stricta* that I have cultivated, obtained from foreign sources, had long, very acute leaf-teeth, similar to those of E167A, and they were usually rather few in number. The upper cauline or lower floral leaves were usually rather narrow, and some had a rather rounded base, but others were truncate. Usually *E. stricta* has a rather large lilac flower, and few short erect branches. These two characters were lacking in E167A. However, they are not always present in *E. stricta*, and Professor W. Rothmaler, to whom I sent plants of the same type as E167A, collected in 1954 from the same locality, considered that they were *E. stricta* var. *parviflora* Sag.

After the publication of Wettstein's monograph, *E. stricta* was widely reported from Britain. In Pugsley's revision, however, *E. stricta* was not accepted as occurring in Britain; plants previously identified as *E. stricta* were referred by him to *E. nemorosa*, *E. confusa* and *E. pseudokermeri*. I find these identifications acceptable, but I believe *E. stricta* has now been found in Britain, both at
Medmenham and at Keston limeworks chalk-pit, Kent; there are specimens at Kew from Keston, collected in 1931 by W. B. Turrill. On a visit there in 1955, I was unable to get into the quarry, but the ground in it and around the edges looked unsuitable for Euphrasias, so there may no longer be any at this locality. I have encountered a few populations of \textit{E. nemorosa} in which there were resemblances to \textit{E. stricta}, and it may be that \textit{E. stricta} was formerly in Britain, or has been introduced from time to time, but has in most places been unable to maintain itself as a distinct entity.

\textbf{II. Comparison of Euphrasia Samples in the Garden in 1954.}

In 1954 an attempt was made to repeat the 1953 experiment on a smaller scale, using different \textit{Euphrasia} populations. The plants were grown at Cambridge, where conditions were apparently less favourable than at Leicester, and very few plants survived. However, a fairly good idea of the characters of the populations was obtained.

The experiment was arranged in randomized blocks, as before; there were five blocks, each consisting of five rows of five plants each. Five populations, of forms thought to be \textit{E. nemorosa}, were used and all were grown on \textit{Plantago lanceolata}. All the plants of one population died; the others were as follows:
E417 between Warslow and Elkstones, Staffs.; long grass and stony patches by roadside on acid moorland but accompanied by some more or less calcicolous plants;

E421 Friday Street, Surrey; sandy field;

E429 West Harling Heath, W. Norfolk; calcareous sandy soil;

E430 Devil's Dyke, Cambs.; on part of chalk dyke levelled in 1943.

The population which survived best was E429, eight plants living until September, of which seven were then pressed; three plants of each of the other populations were also pressed in September. As in the previous experiment, leaves and flowers were mounted on tape. Only one leaf was collected from each plant; the aim was to take 'bract 4' (defined on p.258) but if this was not possible the nearest leaf in good condition was collected.

In general appearance three populations (E417, E421 and E430), although distinguishable by eye, were much like E210, E211 and E215 grown in 1953. E417 showed the closest resemblance to the 1953 samples just mentioned, being very vigorous and rather large-leaved. E430 and E421 were rather smaller plants with narrower leaves, E421 differing from E430 in that its branches diverged for a greater proportion of their length and became vertical only at the tips. E429 had smaller leaves and thinner stems than the other three populations, and it was not so tall as E417. Most of the E429 plants were quite luxuriant, however, for they produced a great profusion of branches; these were
usually widely spreading and somewhat flexuous. A photograph of the most vigorous plant of E429 is shown in Fig. 100, p. 286.

The leaves of the four populations are shown in Fig. 99. Those of E417 are broader, more deeply toothed and apparently more mature than those shown for the similar

\[ E417 \text{ (1954).} \quad E421 \text{ (1954).} \]

\[ E429 \text{ (1954).} \]

\[ E430 \text{ (1954).} \]

\[ E608 \text{ (1956). Left - 'leaf 2'. Right - 'bract 4 or 5'.} \]

**FIG. 99:** Leaves of *Euphrasia* cultivated in the garden in 1954 (approximately 'bract 4') and 1956.
plants grown in 1953 (Flora, 92 & 93, pp. 269-270). Those of
E430, on the other hand, are of a less mature type than
even those of E429 (1951). However, type ' plant at 9'
leaves being the same in both. The same conditions
prevail in the intermixture of both species, and the
Breeds have shown that the type which are identical with
E. confusa from the west of England, while there, in
addition, there are many populations which, although

FIG. 100: Plant of E429, 18 September 1954.
plants grown in 1953 (Figs. 92 & 93, pp.269-270). Those of E430, on the other hand, are of a less mature type than even 'leaf 2' of E215 in 1953. However, some 'bract 4 or 5' leaves of plants of E. nemorosa (E608), which came from the same locality as E430 and were grown in 1956 under the same conditions, were similar to those of E417. The leaves of E421 were also similar to those of E417, and the leaves of E429, though smaller, were of essentially the same type.

The flowers were largest in E417 and smallest in E430, and the flowers of E429 differed from those of the other samples in being lilac instead of white. The flower-shape was different in each sample.

Wild Populations of the 1954 Culture.

The differences between the 1954 samples in cultivation were mostly similar to differences seen in the wild plants from the same localities.

E429, with its small leaves, short internodes, ascending main stem, and abundant, often flexuous, branches, looked very much like E. confusa. Wild plants from the Breckland locality of E429 also resembled E. confusa in their small leaves and flexuous branches but, unlike the cultivated plants, they had the erect main stem and long internodes of E. nemorosa. Later excursions to the Breckland have shown that plants which are identical with E. confusa from the West of England occur there; in addition, there are many populations which, although
similar to *E. confusa*, show some resemblance to *E. nemorosa*. If considered in relation to *E. pseudokerneri* and *E. nemorosa*, their nearest relatives occurring in East Anglia, all these Breckland populations could together be regarded as a distinct taxon, but it would be difficult to regard all of them as *E. confusa*.

III. Comparison of Samples of *E. anglica* Grown in the Garden.

The cultivation of two samples of *E. anglica* at Leicester in 1953 has been described in full on pp.133-4. There were ten "whalehide" pots for each sample, and each pot was planted with two *Euphrasia* seedlings and a plant of *Luzula campestris*. The pots of each sample were arranged in a row, the two rows being side by side. The seed of the two samples (El68 and E219) was collected from two localities threequarters of a mile apart in Charnwood Forest, Leicestershire.

The two populations differed in habit, El68 having shorter internodes than E219. The average height after pressing of the 13 surviving plants of El68 was 7.2cm. (range: 3.3 - 9.3cm.), and that of the 16 survivors of E219 was 11.1cm. (range: 4.4 - 16.5cm.).

'Bract 6', defined in the same way as 'bract 4' (p.258), was mounted for each plant (Fig. 101, p.289). In El68 the bracts did not attain so large a size as in E219, and they also had fewer teeth, the greatest number of teeth

on a side of 'bract 6' averaging 5.0 in E168 (range: 4 - 6) and 6.2 in E219 (range: 5 - 7).

The flowers of E168 were smaller, but more uniform in size, than those of E219, and they also differed from them in shape (Fig. 102, p.290). Some measurements were made on the flowers, the unit of measurement being \( \frac{1}{2} \) mm. The depth of emargination of the midlobe of the lower lip averaged 2.7 in E168 and 4.7 in E219. In E168 the width of the midlobe averaged 9.0 at the base and 11.4 at the widest

point, while in E219 it averaged 10.4 at the base and 15.6 at the widest point.

The differences shown by these two populations are quite considerable in view of their separation in the wild by only three-quarters of a mile. However, E. nemorosa was growing with E. anglica (E219), but not with E168. It has been shown (Yeo, 1956) that introgression appears to take place from tetraploid species into diploids, and it is, therefore, possible that this has been taking place at the locality of E219. Furthermore, another population of E. anglica, growing some miles away from E168 and also unmixed with any other species, was cultivated in the greenhouse with E168 and, although found to differ from it in flower-shape, leaf-shape and habit, the differences were never greater, and were mostly smaller, than those between E168 and E219.
GENERAL DISCUSSION

The taxonomic situation in Euphrasia, outlined in the Introduction (pp.iv-vi), is that there are a great many species distinguished by small and somewhat inconstant differences, and that some of the species are widespread but many are endemic to quite small areas. Observations on hybrids indicate that each species can probably interbreed freely with several of those most closely similar to it, but is increasingly cut off from direct gene-exchange with other species as their morphological difference increases.

The chief biological peculiarity of the northern Euphrasias is that they are semiparasitic. The question arises whether this parasitism is at all specific, and if so whether it could be a cause of spatial ecological isolation, allowing numerous more or less interfertile species to remain distinct. However, the work that I and my predecessors have done makes it clear that Euphrasia species can parasitize a wide range of hosts, so that they cannot have been differentiated and maintained as species by the evolution of different parasitic specializations. The association of E. micrantha with Calluna and Erica, which has been mentioned, is not invariable, and other species can serve as hosts to E. micrantha in cultivation, so that parasitic specialization is apparently not the reason for the association. No other species has been found to show an association of this kind.
The discovery of only two chromosome numbers in 21 species indicates that the numbers are stable, and are therefore not responsible for genetical isolation between closely related species. They merely divide the species into two groups, diploid and tetraploid, which cannot merge. Apomixis has also been ruled out as an isolating mechanism.

Another possible cause of the evolution of new forms of *Euphrasia* is hybridization. This, however, can only operate after a primary differentiation of forms has taken place. One process, involving hybridization, which can account for the origin of new species has been described for *Euphrasia* (Yeo, 1956); one parent in this process is diploid and the other tetraploid, while the new form is a diploid, isolated ecologically from the parent diploid. This method only operates in certain circumstances, but it shows that hybridization can play a part in the origin of new forms.

A combination of geographical and ecological isolation is regarded by Stebbins (1950, p.204) as the most common species-isolating mechanism in plants. Other possible causes of primary speciation in *Euphrasia* having apparently been ruled out, one is led to regard spatial isolation as the most likely cause. The reason for searching for other causes in *Euphrasia* is not so much that the species are poorly-differentiated and interfertile, as that there are so many species of this type. There must be many pairs or
small groups of species as poorly-differentiated as those of *Euphrasia*, but large groups are probably uncommon when sexual reproduction and normal chromosome behaviour are the rule.

Regarding speciation by spatial isolation, this could only be effective in the presence of variability and a selection pressure in favour of differentiation. That variability exists within populations can be seen from the illustrations of flowers accompanying the account of artificial hybrids and the account of comparisons between populations of the same species (e.g. Fig. 73, p.221, and Fig. 94, p.272). *Euphrasias* are highly gregarious, but the distribution within populations is often patchy, even where there is little or no perceptible variation in the habitat, which suggests an acute sensitivity to environmental conditions. Such a sensitivity might provide a selection pressure in favour of ecological specialization. Though the differentiation of *Euphrasia* species is apparently not the result of host-specialization, it is clearly to a large extent ecological. Many of the specific characters are adaptations to biotic factors; thus species growing in closely-grazed turf, such as *E. anglica* and *E. confusa*, are dwarf and bushy, those found in rather longer grass, such as *E. nemorosa*, are taller, with branches originating higher up the stem, and the hayfield species, *E. rostkoviana* and *E. brevipila*, are tallest of all. Edaphic adaptations presumably do not show themselves in the outward morphology
of the plants, but the stout, condensed habit of the maritime *E. occidentalis*, and to some extent the different dates of flowering of the various species, may be regarded as climatic adaptations. Thus the origin of many, or perhaps of all, species may be explained by an inability to colonize certain habitats without specialization. A narrow ecological tolerance would have enabled many species to exist in a fairly small area, and would have required the evolution of localized species to colonize geographically localized habitats, or those habitats from which the characteristic species had been cut off by geographical accident. It is to be expected that, accompanying the ecologically adaptive differences, a number of more or less non-adaptive differences should have arisen to characterize the species. But there are also differences between species in flower-size, and hence in breeding system; these differences are presumably adaptive, but they are probably not ecologically adaptive. In this connection it may be remarked that the North American species are particularly small-flowered, being about 2.5 to 7mm. in corolla length; the largest-flowered of these species is *E. frigida*, which also occurs in Europe. The corolla length in *Euphrasia* species in Europe ranges from 5 to about 12mm., except in *E. bottnicoua*, which is related to some very small-flowered American species, and has flowers 2.5 to 3mm. long. It is difficult to see any correlation between flower-size and any other aspect of the biology of Euphrasias, and hence
difficult to see in what way variation in flower-size can be adaptive. There is no evident reason why one species should be adapted to a greater degree of outbreeding than another. One possible advantage of small flower-size might arise when a new species comes into contact with a well-established large-flowered one; the spread of the new species through the area of the old might be facilitated if small flower-size gave the new species a high degree of genetical isolation.

Examples of the few genera which resemble Euphrasia in having a multiplicity of poorly defined species, which are interfertile, are Aster in North America and Aquilegia. However, both genera differ from Euphrasia in being perennial. Aquilegia is also distinct in that its main specific differences are floral, and that natural hybridization is not common in this genus. As far as I know, there has not been much experimental study of Aster.

In addition to the problem of speciation in Euphrasia, it is difficult to see how the Euphrasia species have gone through their migrations without losing their identity. It is common to find two Euphrasia species growing in different but adjacent habitats; where the habitats meet, the species hybridize, and the size of the zone of interbreeding depends on the area of the intermediate habitat, or the presence of other suitable habitats, such as roadsides, within reach of both species. One would expect that, in a time of climatic change and changing habitats accompanied
by migration of *Euphrasia* species, many hybrid populations would arise, and that when conditions again became more stable a patchwork of different forms would remain, made up of surviving populations of the species and their hybrids. In fact this is not the case; populations of hybrid origin, not growing with the parents, apparently occur, but they are rather rare, and are usually small in extent. It appears therefore that certain factors are operating to maintain the integrity of the species in spite of hybridization, such as close genetic linkages between the genes governing a number of important specific characters. The range of variation seen in wild hybrid populations does not suggest that these linkages exist, but to investigate the matter properly it would be necessary to grow artificial hybrids for two or three generations and to study them thoroughly morphologically. Such linkages might be maintained by chromosomal interchanges or inversions, which a cytological investigation might be able to reveal.

Though past hybridization seems to have failed to disrupt the species, it may have contributed to their variability at the present time. It is usual to find some variation in flower-shape within populations, and some between neighbouring populations of the same species. Occasionally, however, one finds very uniform populations (e.g. *E. nemorosa* (El51A), described in the account of the garden trial at Leicester on pp.261-274); in each of two other cases where a uniform population was found, an
apparently identical population was found at a locality about twelve miles away.

It is probable that the variation in flower-shape existing in populations is accompanied by inherited variation in other characters, although it is not often possible to detect this with certainty, even in cultivation. The limited outbreeding which probably takes place in nature may be sufficient for the maintenance of this variation within populations, while some of the differences between populations may be the result of adaptation to local conditions. Adaptation probably takes place readily, because Euphrasias usually grow in stable habitats, and the greater part of any population must grow on the same ground from year to year. The differences in survival, found between populations under uniform conditions in the garden at Leicester, suggest that physiological adaptations to local conditions can in fact arise. Euphrasias are unlike the annuals of ruderal habitats, which may not grow on the same ground from year to year and which require phenotypic plasticity rather than the evolution of close adaptation to their environment.

The variability and lack of genetic isolation of northern hemisphere Euphrasia species suggest that this part of the genus has considerable potentiality for evolution, and that much of the evolution of the genus has been recent. The idea that many species have evolved recently is supported by the occurrence of endemics in the
British Isles, such as *E. pseudokerneri*, *E. cambrica* and *E. rivularia*, which are not likely to have survived the glacial period; these species, together with *E. anglica* and *E. confusa*, which are particularly adapted to existence in grazed turf, are likely to have arisen in the last interglacial period or in the postglacial period. However, the wide distribution of the genus in the northern hemisphere indicates that it must have been there for a long time, and some of its more wide-ranging species must also be fairly old.

I have no information about distribution of seed by non-human agency, but inadvertent human agency seems to be quite effective. A taxonomic revision of the North American species, in which Mr. P. D. Sell and I are engaged, together with the observations of Fernald (1915), indicates that four European species have probably been introduced by man into North America.

Some parts of the morphological classification of British Euphrasias by Pugsley are supported by my work. Thus the whole of the Series Hirtellae proves to be diploid, while the other groups are tetraploid. *E. pseudokerneri* and *E. occidentalis* are very different in habit (Fig. 74, p.227) but similar in leaf shape, while *E. scotica* differs more from *E. pseudokerneri* in leaf shape (Fig. 72, p.220). Pugsley (1930) placed *E. pseudokerneri* and *E. occidentalis* together in Series Nemorosae, and *E. scotica* in Series Latifoliae. The plants of both Series show a considerable
range of habit, and Pugsley must have relied heavily on leaf characters in classifying the species. The fact that the artificial hybrid E. pseudokerneri x E. occidentalis was more fertile than E. pseudokerneri x E. scotica indicates that Pugsley's assessment of affinity on morphological grounds was in accord with the genetic affinity of the species.

The genus Euphrasia probably originated in the southern hemisphere and appears to have undergone considerable radiation there. The South American species are related to those of Australasia and not to those of North America. The range of leaf shapes in Australasia includes broad types similar to those of Subsection Ciliatae and narrow, finely-toothed types like those of Subsection Angustifoliae. Du Rietz (1931) has shown how there is a gradual transition in the West Pacific area from southern forms to the species of the Japanese Series Japonicae and Alpicalae. These Japanese groups likewise include broad-leaved and narrow-leaved forms, which might have given rise to Subsections Ciliatae and Angustifoliae respectively. However, if the Angustifoliae arose from a Japanese group, it is curious that they are now found only in Europe and Turkey.

The behaviour of the chromosomes in the triploid E. anglica x E. micrantha suggests that the tetraploid members of Subsection Ciliatae are allotetraploids and contain one genome derived from the diploid members of the Subsection, and one derived from some other diploid group.
In *E. salisburgensis* var. *hibernica* x *E. occidentalis*, many of the pollen mother cells showed about 22 univalents at meiosis, suggesting the possibility that one genome, but not the other, in the tetraploid members of Subsection Ciliatae is closely related to one of the two genomes in Subsection Angustifoliae.

It is sometimes the case that investigations of the kind described here lead to possible modifications in the taxonomy of the group. In the present work, three minor instances of this have arisen. The garden trial of *E. nemorosa* led me to an understanding of variation which I was able to apply in subsequent field work, and from this I have concluded that Pugsley's varietal subdivision of *E. nemorosa* cannot be maintained. Forms such as he describes can be found, but they differ in different localities and evidently have no common origin, while the descriptions cover only a small part of the variation found in the species. The same experiment also assisted in the recognition of *E. stricta* as a British plant (p.282-283). Thirdly, it was suggested (p.144) that the Series Hirtellae, being diploid unlike the other Series grouped with it in Subsection Ciliatae, should be raised to the rank of Subsection. However, there is such a close approach in morphology between the diploid and tetraploid groups that it seems inadvisable to do this.

My work constitutes a beginning of a knowledge of the biology of the species of *Euphrasia* in relation to evolution
and, although it has not advanced far enough for positive suggestions to be made to account for the taxonomic difficulties presented by *Euphrasia*, it does rule out certain possibilities. The taxonomy of *Euphrasia* must continue to rest largely on morphology, supported by field observation. It seems clear, from the herbarium material and wild populations that I have seen, that Pugsley's "Revision" was a great advance in the taxonomic understanding of British *Euphrasias*, and it seems to indicate a deep taxonomic insight on his part. However, Pugley's concepts of *E. borealis* and *E. hirtella* appear to need revision, but it is difficult to produce a better treatment. As already mentioned, Pugsley's subspecific taxonomy of *E. nemorosa* does not seem valid; and the same applies to *E. occidentalis*.

Future work on *Euphrasia* might include further investigation of parasitism, including a more thorough comparison of host range and suitability for different *Euphrasia* species, more detailed cytological and genetical work, if the material allows it, and the counting of the chromosomes in the divisions of the genus which have not been investigated. It might also be possible to find out more about which substances the *Euphrasia* plants obtain from their hosts, and about the relative importance of these substances.
Localities of Euphrasia Specimens.

The serial numbers represent collections of seed, living plants, herbarium specimens, or combinations of these. The Euphrasias were collected and determined by me unless otherwise stated.

Nearly all gatherings of which wild-collected herbarium material exists are represented in my own herbarium and that of the University of Leicester, and many are also in the University Herbarium, Cambridge. Cultivated specimens are either in my herbarium or that of the University of Leicester.

\[ E \]
23 pseudokerneri, Box Hill, Surrey, v.c. 17. 29/8/51.
35 occidentalis, Barton-on-Sea, S. Hants., v.c. 11.
11/9/51.
42 pseudokerneri, Holywell Mound, S. Lincs., v.c. 53.
11/10/51.
53 brevipila, Glen Orchy, Argyll, v.c. 98. 5/9/51.
(Coll. T. G. Tutin, 51130).
60 pseudokerneri, as E42. 8/11/51.
66 pseudokerneri, as E23. 15/4/52.
67 anglica, Box Hill, Surrey, v.c. 17. 15/4/52.
70 pseudokerneri, as E42. 29/4/52.
71 anglica, Holmsley, S. Hants., v.c. 11. 3/5/52.
72 nemorosa, Forest Lodge, S. of Holmsley, S. Hants.,
v.c. 11. 3/5/52.
73 occidentalis, as E35. 3/5/52.
74 nemorosa, Cherry Hinton, Cambs., v.c. 29. 8/5/52.
75 pseudokerneri, Gog Magog Hills near Cherry Hinton, Cambs., v.c. 29. 8/5/52.
76 anglica, as E67. 25/5/52.
77 pseudokerneri, as E23. 25/5/52.
90 brevipila, Capel Curig, Caerns., v.c. 49. 22/6/52.
100 curta var. rupestris, Cwm Idwal, Caerns., v.c. 49. 23/6/52.
107 confusa x micrantha, Capel Curig, Caerns., v.c. 49. 24/6/52.
112A micrantha, Capel Curig, Caerns., v.c. 49. 24 & 25/6/52.
120 scottica (customarily spelt 'scotica' by British authors), Cwm Idwal, Caerns., v.c. 49. 27/6/52.
123 rivularis, Cwm Idwal, Caerns., v.c. 49. 27/6/52.
129 nemorosa, Waltham Quarry, Leics., v.c. 55. 10/7/52. (Coll. E. K. Horwood).
134 nemorosa, Bedford Purlieus, Northants., v.c. 32. 10/7/52.
135 & 135A anglica, White Hill near Copt Oak, Leics., v.c. 55. 14/7/52 & 27/8/52.
139 anglica, Foulden Common, W. Norfolk, v.c. 28. 17/7/52.
143 pseudokerneri, Risby Poor's Heath, W. Suffolk, v.c. 26. 16/7/52.
149 nemorosa, Banstead Downs, Surrey, v.c. 17. 23/7/52.
150 anglica, as E67. 24/7/52.
151A nemorosa form, Box Hill, Surrey, v.c. 17. 24/7/52.
154 nemorosa, Pray Heath, Worplesdon, Surrey, v.c. 17. 25/7/52.
anglica, Mickleham Downs, Surrey, v.c. 17. 26/7/52.


nemorosa, Mere Road, Saltby, Leics., v.c. 55. 13/7/52. (Coll. E. K. Horwood).

nemorosa, Watlington Hill, Oxon., v.c. 23. 12/8/52.


stricta, with El67A.

anglica, Ling Hill, Charnwood Forest, Leics., v.c. 55. 13 & 27/8/52.

confusa, Withypool Hill, Withypool, S. Somerset, v.c. 5. 19/8/52.

confusa, West Water valley, Withypool Hill, S. Somerset, v.c. 5. 19/8/52.

confusa, S. side of Withypool Hill, S. Somerset, v.c. 5. 19/8/52.

confusa, N. of Landacre Bridge, near Withypool, S. Somerset, v.c. 5. 19/8/52.

anglica, Withypool, S. Somerset, v.c. 5. 20/8/52.

confusa, heath N.W. of Withypool, S. Somerset, v.c. 5. 20/8/52.

micrantha, heath N.W. of Withypool, S. Somerset, v.c. 5. 20/8/52. A single triploid plant.

anglica x micrantha, with El85A. Diploid form.

occidentalis, Rame, near Plymouth, E. Cornwall, v.c. 2. 22/8/52.

nemorosa form, Roborough Down, near Plymouth, S. Devon, v.c. 3. 22/8/52.

vigursii, with El96.

salisburgensis, between Salzburg and Eisriesenwelt, Austria. August 1952. (Coll. J. J. Yeo).
208 confusa, foot of Thorpe Cloud, Dovedale, Derby, v.c. 57. 13/9/52.

209 confusa, Lover's Leap, Dovedale, Derby, v.c. 57. 13/9/52.

210 nemorosa, as E129. 18/9/52.

211 nemorosa, The Drift, Saltby, Leics. - S. Lincs., v.c. 55-53. 18/9/52.

215 nemorosa, as E134. 18/9/52.

219 anglica, Swithland, Charnwood Forest, Leics., v.c. 55. 19/9/52.

226 pseudokerneri, as E23. 24/9/52.


240 anglica, as E168. 28/2/53.

245 brevipila, near Waterfall, Staffs., v.c. 39. 22/6/53.

247 borealis, W. of Bowes, N.W. Yorks., v.c. 65. 28/6/53.
<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
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<tr>
<td>254</td>
<td><em>brevipila var. notata</em>, Lawers, Mid-Perth, v.c. 88. 30/6/53.</td>
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<td>256</td>
<td><em>brevipila var. notata</em>, Lawers, Mid-Perth, v.c. 88. 1/7/53.</td>
</tr>
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<td>261</td>
<td><em>scottica</em> (customarily spelt 'scotica' by British authors), shore of Loch Tay, Lawers, Mid-Perth, v.c. 88. 2/7/53.</td>
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<tr>
<td>262A</td>
<td><em>brevipila var. notata</em>, by Loch Tay, Lawers, Mid-Perth, v.c. 88. 2/7/53.</td>
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<td>304</td>
<td><em>brevipila var. reayensis</em>, Farr Bay, Bettyhill, W. Sutherland, v.c. 108. 8/7/53.</td>
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<tr>
<td>308</td>
<td><em>brevipila var. reayensis</em>, as E304. 8/7/53.</td>
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<td>311</td>
<td><em>marshallii</em>, Bettyhill, W. Sutherland, v.c. 108. 9/7/53.</td>
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<tr>
<td>325</td>
<td><em>brevipila</em>, Durness, W. Sutherland, v.c. 108. 10/7/53.</td>
</tr>
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<td>329</td>
<td><em>borealis</em>, W. of Bowes, N.W. Yorks., v.c. 65. 12/7/53.</td>
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<tr>
<td>401B</td>
<td><em>nemorosa</em>, as E167A &amp; B. 19/8/53.</td>
</tr>
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<td>419</td>
<td><em>nemorosa</em>, as E154. 26/9/53.</td>
</tr>
<tr>
<td>421</td>
<td><em>nemorosa</em>, Friday Street, Surrey, v.c. 17. 26/9/53.</td>
</tr>
<tr>
<td>424</td>
<td><em>suecica</em>, Darss, Prerow, Mecklenburg, Germany. 1953. (Coll. W. Rothmaler). I am not properly acquainted with this species, which is related to <em>E. brevipila</em>.</td>
</tr>
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429 cf. confusa, West Harling Heath, W. Norfolk, v.c. 28. 18/4/54.
430 nemorosa, Devil's Ditch, Cambs., v.c. 29. 18/4/54.
469B nemorosa x pseudokerneri, as EI 151A. 14/8/54.
474 nemorosa, Mickleham, Surrey, v.c. 17. 16/8/54.
493 anglica, Larling Heath, W. Norfolk, v.c. 28. 5/9/54.
507 nemorosa, as EI 430. 18/9/54.
514 tatrae, Tatra Mountains, Poland. 1954. (Coll. Z. Radwanska-Paryska, per Warsaw University Botanic Garden). Not determined by me.
520 rostkoviana, Poland. 1954. (Coll. Institut de Pharmacognosie, Lodz, Poland).
530 rostkoviana, Friuli, Italy. 1954. (Coll. Botanic Garden of the Technical Institute, Udine, Italy).
559 hirtella, Roc'h Trevezel, Brittany, France. 15/7/55.
561 occidentalis, Le Diben, N. of Morlaix, Brittany, France. 16/7/55.
571 occidentalis, Camaret, presqu'île de Crozon, Brittany, France. 19/7/55.
594 pseudokerneri, Deacon Hill, Pegsdon, Beds., v.c. 30. 21/8/55.
601 confusa, Glencorse Reservoir, Roslin, Midlothian, v.c. 83. 12/9/55.
603 scottica (customarily spelt 'scotica' by British authors), as EI 601. 12/9/55.
604 micrantha, near West Linton, Midlothian, v.c. 83. 12/9/55.
606 pseudokerneri, Devil's Ditch, Cambs., v.c. 29. 25/9/55.
608 nemorosa, as EI 430. 25/9/55.
616 anglica, Devon, v.c. 3 or 4. 1955. (Coll. S. M. Walters).

622 stricta, Poland. 1955. (Coll. Institut de Pharmacognosie, Lodz, Poland).

623 brevipila, Germany. 1955. (Coll. Greifswald, Agro-biological Institute, as E. officinalis).

649 anglica, Ucheldref, E. of Pentraeth, Anglesey, v.c. 52. 20/8/56.

650 nemorosa, near Llanfechell, Anglesey, v.c. 52. 20/8/56.

659 scottica, as E120. 21/8/56.

663 anglica, between Felyn Hen and Rhyd-y-Groes, near Bangor, Caerns., v.c. 49. 22/8/56.

699 pseudokerneri, as E606. 12/6/57.

701 nemorosa, as E430. 12/6/57.
ACKNOWLEDGMENTS

The work described here owes its existence to Professor Tutin, under whose guidance it was begun and continued, and to the University College (now University) of Leicester. In addition to the invaluable contributions of the Departments of Botany at Leicester and Cambridge, I was assisted by my fellow research students at Leicester and by numerous people who have collected specimens for me, especially Professor Tutin and Mr. E. K. Horwood. Dr. E. F. Warburg gave me the benefit of his taxonomic opinion, and also took me to the Euphrasia locality at Medmenham (E167A and B). Two important contributions by Mr. Wilkins have been mentioned in the text.

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Summary of Thesis by P. F. Yeo, entitled
"Experimental Taxonomy on the Genus Euphrasia".

Euphrasias are semi-parasitic annuals whose roots attach themselves to the roots of other plants. Because of their similarities, the many species present taxonomic difficulties.

Development and parasitism were studied by cultural means. Seed germinates in spring and the plants flower in summer. Euphrasias can produce seed in isolation; when grown together they parasitize one another; in either case they are usually less vigorous than Euphrasias grown in pots in their natural turf, or planted as seedlings with a chosen host-plant. Some hosts are more favourable than others. With garden cultivation many plants became extraordinarily vigorous.

Five related species have diploid chromosome-numbers, but the majority are tetraploid; one wild triploid was found.

The possibility of cross-pollination increases with flower-size, but even the largest flowers have a good chance of eventual self-pollination. The insects seen visiting the flowers were chiefly Apidae and Syrphidae. Presumed Euphrasia hybrids are frequent in nature. Many were found in the progeny of open-pollinated Euphrasias in cultivation, including two from a very small-flowered species.
Apomixis has not been detected. Self-pollination gave approximately normal seed-yields, as did the crossing of species alike in chromosome-number. Cross-pollination between diploids and tetraploids gave no normal seed.

Closely-related parents gave hybrids more or less normal in fertility, but sterility increased with increasing parental dissimilarity; this also applied to F2 hybrids.

Several E. nemorosa populations, one of E. stricta and two of E. anglica were grown in the garden and compared. Some populations showed conspicuous differences, others slight but statistically significant floral differences.

Geographical and ecological isolation are probably the chief agents of speciation in Euphrasia, as in most flowering plants. Explanations of the poor definition of the species are tentative; Euphrasia is in an active phase of evolution. The taxonomy of the British species, as left by Pugsley, is largely accepted. Suggestions for future work are made.
THE CYTOLOGY OF BRITISH SPECIES OF EUPHRASIA

By P. F. Yeo

University Botanic Garden, Cambridge*

The counting of the chromosomes of Euphrasia species was undertaken in connection with an investigation of the breeding system and fertility relationships of the British species.

PREVIOUS WORK

There has been little previous work on the cytology of European species of Euphrasia, and as far as is known to the author, none of this has been on British material.

Four species were counted by von Witsch (1932); they were as follows: E. minima Jacq. subsp. minima (not British), n = 22; E. rostkoviana Hayne subsp. rostkoviana, n = 11; E. montana Jord., n = 11; E. salisburgensis Funck, n = 22. In addition A. & D. Löve (1948) report that Sörensen and Westergaard found n = 22 in E. frigida Pugs., in Greenland. Maude (1939) records 2n = 44 for "E. confusa Pugs. (minima auctt. angl.) von Witsch, 1932," but as shown above, it was E. minima Jacq. that von Witsch counted. Finally, Tischler (1950) gives the entry "E. brevipila Burn. et Gremli (E. montana Fries) n = 11, von Witsch (1932)." This must refer to von Witsch's count of E. montana Jord. All the names used by von Witsch are current names for well-known species and there seems no reason to suppose that he cited his authorities wrongly.

METHOD

(1) Material

The use of root tips was not attempted. Root tips would be very difficult to obtain from wild plants owing to the slenderness of the roots, and their consequent fragility. They could, however, have been obtained from young seedlings, but when this work was started the few seedlings available were wanted for cultivation. Work was therefore confined to the use of pollen mother cells.

(2) Collecting

Observations on cultivated plants showed that flowers at successive nodes open at intervals of three or four days, or, in the case of E. pseudokernerii, five to seven days. Meiosis thus occurs in any one spike with this frequency, and takes place roughly four or five nodes above that of the flower open at the time. It should thus occur periodically from about a fortnight before flowering starts until a fortnight before it ceases. Actually it probably ceases earlier, because flowers at the last few nodes open at shorter intervals, and there may be flowers open at two or three successive nodes simultaneously, so that just before it stops flowering the plant becomes very floriferous. Meiosis seems to be farther advanced when flowering is about to end, since it is found to have taken place in smaller flower-buds than usual. For fixing, therefore, good solid shoot apices with plenty of young bracts have to be chosen. These occur on the more luxuriant plants. If the plants are growing under unfavourable conditions, flowers may be produced at only three or four nodes, and then meiosis may be over when the first flower opens. As a result of this, material of E. cambrica obtained in 1952 proved completely useless.

* The work reported in this paper was carried out during the tenure of a Research Scholarship at the University College of Leicester.
The time taken by meiosis is evidently less than the interval between the opening of the flowers, and thus one is not bound to find meiosis in a particular spike, even if it has not occurred in that spike for the last time. In addition, therefore, to selecting spikes of the kind described, an adequate quantity must be collected. The same fact also makes obtaining counts a time-consuming process, though the chance of getting a count is increased by the fact that the two pairs of anthers of each flower are at different stages, and also that various stages are sometimes found in the same anther.

(3) **Fixing**

Buds were fixed in acetic acid and ethyl alcohol, mixed in the proportions 1:3, or more usually, in acetic acid, alcohol, and chloroform in the proportions 1:3:4. In the case of wild plants, buds were fixed either at the end of a day's collecting, the plants having been kept in the vasculum, or as soon as the gathering was complete, or they were cut off the plants in situ. The last method was always used for cultivated plants.

(4) **Storage**

Fixed material was preserved in a refrigerator at about $-15^\circ$C., as described by Davies (1952). The oldest material used had been stored in this way for six or seven months.

(5) **Staining and Mounting**

The acetocarmine squash method was used, iron alum or iron acetate being used as a mordant. For permanent preparations triacetin was used. A drop is placed at the edge of the coverglass and as the acetic acid of the stain evaporates the triacetin takes its place. This is relatively involatile and allows the preparation to be studied at leisure without it drying up, while the removal of the carmine eliminates the danger of the cytoplasm becoming stained. After the removal of excess triacetin the coverglass is ringed with "Euparal".

**Chromosome Counts**

These are based on any stage of meiosis at which the chromosomes could be counted. In *Euphrasia* prophase does not yield satisfactory counts, though at late diakinesis counts can occasionally be obtained. Metaphase and early anaphase of the first division yield counts most easily. In tetraploids (n=22) the later stages can rarely be counted reliably; of these, late anaphase of the first division (anaphase I) is the most likely to give a count. In diploids (n=11), however, late anaphase I, metaphase II, and anaphase II can frequently be counted without difficulty.

There was not time to work on all the material available, and species on which no counts had been made previously (in particular British endemics) were given preference.

All the plants were determined by the writer. In some cases the fixed specimens were kept separate from others in the same gathering, and these are either in the writer's herbarium, or at the Department of Botany, University College of Leicester. Where the fixed individuals were not separated, the gathering concerned is represented in both these herbaria, and a number are also at the Cambridge Botany School.

(1) *E. micrantha* Reichb. Wild material fixed at Withypool, S. Somerset, in August 1952 proved to have finished meiosis. Seeds from this locality (specimen No. E185A) were grown in 1953, but too few spikes were fixed. As a result no meiotic metaphases were obtained, but diakinesis was observed which, though not quite unambiguous, can be interpreted as showing 22 bivalents.

(2) *E. scottica* Wettst. Material from Lawers, Mid Perth (E261) shows n=22.
(3) *E. foulaensis* Towns. A count was obtained from cultivated material (E227) showing n=22. The seed came from live plants sent from N. Uist, Outer Hebrides, in 1952, by Mr. R. G. West.

(4) *E. marshallii* Pugsley. E311, Bettyhill, W. Sutherland, showed n=22.

(5) *E. curta* Fries var. *rupes* Pugsley. Cultivated material (E100) showed n=22. E100 was collected in Cwm Idwal, Caernarvon, in June 1952, and consisted of herbarium specimens of *E. cambrica* Pugsley, and turves. The only plants that persisted in the turves were *E. curta* var. *rupes*, which was not generally in flower at the time the turf was collected. Seeds were collected from the plants in the turves and grown in 1953. The plants gave a small number of buds from which the count was obtained. E100 therefore consists of herbarium specimens of wild *E. cambrica*, and cultivated *E. curta* var. *rupes* from which the chromosome number was obtained.

(6) *E. occidentalis* Wettst. n=22, from E192, Rame, E. Cornwall.

(7) *E. nemorosa* (Pers.) H. Mart, emend. Löh. Two counts were obtained, both of n=22, one from E134, Bedford Purlieus, Northants., the other from E149, Banstead Downs, Surrey.

(8) *E. confusa* Pugsley. n=22, E175, from Withypool, S. Somerset.

(9) *E. pseudokerneri* Pugsley. n=22; plants from the Devil’s Dyke, near Brandon, W. Norfolk (Plate 10, fig. 3) (E136), and Risby Poor’s Heath, W. Suffolk (E143).

(10) *E. brevipila* Burnat & Gremli. n=22, E245, Sparrowlee Halt, near Waterfall, Stafford. In one cell observed, there is an indication that one pair of chromosomes is represented by two univalents instead of a bivalent, but this is not certain.

(11) *E. brevipila* var. *notata* Pugsley. Two lots of material (E254 and E256) from colonies about half a mile apart at Lawers, Mid Perth, were worked on. In both, n=22 was observed, but some buds showed 2n=44+1, the extra chromosome appearing as a univalent, most frequently either at the edge of the metaphase plate, or well away from it. The occurrence of an extra univalent can be accounted for by supposing that, in one of the parents of an individual possessing it, there occurred a failure of disjunction at meiosis, and that as a consequence of this both members of the pair went to one pole at anaphase, giving pollen grains with twenty-three chromosomes, instead of twenty-two. No anaphases showing evidence of this unsymmetrical distribution were seen, but pairing failure in one pair of chromosomes at metaphase was rather frequent. Plate 10, fig. 4 shows a metaphase in E254 with twenty-one bivalents and three univalents. This has the extra chromosome and shows the pairing failure that probably gives rise to it. It will be noted that all three univalents are of approximately the same size, and that they are about as large as the largest chromosomes among the bivalents. That these chromosomes were univalents was confirmed by Dr. J. R. S. Fincham, upon inspection of the photographic negatives. The only suggestion that the two univalents are members of a bivalent that have separated early is that they are in every case near one another. Dr. Fincham pointed out, however, that as the univalents are almost always found at the edge of the metaphase plate they are probably forced into this position by lack of a spindle attachment, and thus have a good chance of appearing in the same part of the cell in the preparation. The shape of these chromosomes indicates that they are true univalents.

In fixing, more than one spike was usually taken from each plant, and all were fixed together. Thus it is not known whether more than one plant in each gathering had the extra chromosome, and, at least in the case of E256, not all the fixed material was worked through. However, the extra univalent was found in both gatherings, and pairing failure was observed in both, being seen several times in E254.

(12) *E. brevipila* var. *reayensis* Pugsley. This form, and *E. brevipila* var. *notata*, are the only European forms with long glandular hairs that are not classified in the Series
The time taken by meiosis is evidently less than the interval between the opening of the flowers, and thus one is not bound to find meiosis in a particular spike, even if it has not occurred in that spike for the last time. In addition, therefore, to selecting spikes of the kind described, an adequate quantity must be collected. The same fact also makes obtaining counts a time-consuming process, though the chance of getting a count is increased by the fact that the two pairs of anthers of each flower are at different stages, and also that various stages are sometimes found in the same anther.

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(12) **E. brevipila** var. *rayensis* Pugs. This form, and **E. brevipila** var. *notata*, are the only European forms with long glandular hairs that are not classified in the Series
Hirtellae, the members of which, as far as is known, are all diploids. However, the question whether var. reayensis shows, in common with var. notata, any irregularities at meiosis, has not been gone into. This was due to the fact that meiosis had finished in a large proportion of the spikes fixed, and work on the material was stopped after a clear count had been obtained, so that few divisions were seen. The count showed \( n = 22 \); the material was from Bettyhill, W. Sutherland (E304).

(13) *E. rivularis* Pugs. Plants dug up in turf in Cwm Idwal, Caernarvon, in June 1952 (E123), were grown in the greenhouse and produced seed. In 1953 the count of \( n = 11 \) was obtained from a plant grown from this seed.

(14) *E. anglica* Pugs. The number \( n = 11 \) was observed in E71, plants dug up as seedlings at Holmsley, S. Hants; E150, Box Hill, Surrey; E157, Mickleham Downs, Surrey; and E168, Charnwood Forest, Leicester.

(15) *E. hirtella* Jord. var. *polyadena* (Gren. & Roux) Pugs. Material from Lawers, Mid Perth (E253), had \( n = 11 \) (Plate 10, fig. 5).

(16) *E. anglica* × *micrantha*. A single individual (E185C) was found on a heath near Withypool, Exmoor, S. Somerset, at a point where the parent species came into contact. The plant resembled *E. micrantha* in habit, small foliage, and dark anthocyanin pigmentation. The flower had no lilac in it, unlike those of the surrounding *E. micrantha*, and was slightly larger than those, with a larger lower lip, in this respect diverging from *E. micrantha* in the direction of *E. anglica*. The foliage had scattered rather long glandular and eglandular hairs. (*E. anglica* has dense long-stalked glands; *E. micrantha* is sub-glabrous). On the main axis it had flowered at five successive nodes, but there had been no development of any of the capsules. The percentage of normally formed pollen grains was investigated by staining with cotton blue in lactophenol. Of 998 grains from one flower 31, or 31 per cent, were normal in appearance.

The shoot apex from the main axis, and one from a branch of this plant, were fixed, and preparations of pollen mother cell meiosis obtained from both. The chromosomes tended to be clumped, a condition met with occasionally in other material, and this made interpretation difficult. It was clear that a number of univalents, up to eleven, was present, and also some bivalents. What appeared to be larger bodies are evidently groupings of two or three bivalents. Interpreting the chromosomes on this assumption it was possible to count most of the bivalents and univalents in four cells, there being a residue of one or two doubtful bodies. These could be interpreted as large univalents and, if this was done, the conclusion was reached that there were eleven bivalents and eleven univalents present. One of these cells is shown in Plate 10, fig. 6. Figures 1 and 2 show interpretative diagrams of two of them. These interpretations were made from the preparations themselves, but they are not certainly correct. In each of the cells illustrated there is, in fact, one body that looks like a multivalent. These are indicated in the diagrams; that in fig. 2 is very suggestive of a trivalent. The interpretations given, however, are the only ones that give a total of 33 chromosomes. Since the basic number is 11, and since the number in this hybrid is certainly in the neighbourhood of 33, an interpretation that gives exactly this number is more likely to be correct than one giving a different number.

The anaphases seen were too obscure to give any information, but at telophase univalents were occasionally present, faintly stained, at the equator. These lagging univalents usually numbered only one or two.

**General Observations on the Cytology**

The chromosomes are of moderate size. The size varies within the complement but in meiotic material it is difficult to describe the differences in detail, as von Witsch (1932)
found. Roughly there are large, small, and intermediate sizes, the latter apparently in the majority. Mostly there is one chiasma per bivalent, but one or more bivalents with two chiasmata occur in most cells, both in tetraploids and diploids. In the tetraploids no indications of multivalent formation were seen.

The occurrence of an extra univalent in *E. brevipila* var. *notata* was not recognized until a fair number of cells had been seen, and it is conceivable that it could have been encountered in previous work and overlooked. However, the fact that it was never detected indicates that among the species in general it is rare, whereas its discovery in two populations of var. *notata* indicates that it is relatively frequent in that form. Its accompaniment by frequent pairing failure in one pair of chromosomes suggests that it may be quite common in *E. brevipila* var. *notata*, at least around Lawers.

**Discussion**

**Relation of Chromosome Numbers to Classification**

The two diploid species which von Witsch (1932) counted, *E. montana* and *E. rosthoviana*, possess long glandular hairs, and fall in the Series *Hirtellae*, a group created by Pugsley (1930), while all the other species previously counted were tetraploids with \( n = 22 \). This suggested that probably all the *Hirtellae* were diploids, and that all the other members of the Subsection *Ciliatae*, together with the Subsection *Angustifoliae*, were tetraploids. The counts obtained in the present work confirm this pattern, as far as the *Ciliatae* are concerned. This is illustrated in the accompanying table.

<table>
<thead>
<tr>
<th>Table of British species of <em>Euphrasia</em> arranged according to Pugsley's classification, with <em>E. minima</em>, showing their haploid chromosome numbers.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SECTION SEMICALCARATAE</strong></td>
</tr>
<tr>
<td><strong>Subsection Ciliatae</strong></td>
</tr>
<tr>
<td><strong>Series Latifoliae</strong></td>
</tr>
<tr>
<td>micrantha</td>
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<tr>
<td>scotica</td>
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<tr>
<td>rhumica</td>
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<tr>
<td>frigida</td>
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<tr>
<td>foulensis</td>
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<tr>
<td>eucarpia</td>
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<tr>
<td><em>Campbelliae</em></td>
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<td>(minima)</td>
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<td></td>
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<tr>
<td><strong>Series Brevipilae</strong></td>
</tr>
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<td><strong>E. salisburgensis</strong></td>
</tr>
</tbody>
</table>

* Chromosome number obtained from non-British material.

These results confirm Pugsley's classification of the long-glandular species in a separate group, the series *Hirtellae*. Previously they were placed partly in the *Grandiflorae* Wettst. and partly in the *Parviflorae* Wettst., both of which groups also included eglandular species. The cytological distinctness of the *Hirtellae* draws attention to the relative isolation of the group. The species have one constant and almost diagnostic character, namely the covering of long glandular hairs. In addition the capsule tends to be short and broad, the calyx and seeds to be similarly proportioned, and the flower to have a porrect lower lip, with the lobes not greatly emarginate or greatly dilated apically, and a broad upper lip. They do not diverge to any great degree from the series *Brevipilae*,...
which, of the other series of the Ciliatae, they most nearly resemble, but they are relatively well characterized morphologically, and stand apart from the other groups, there being no borderline species. Unlike some groups, the Series Hirtellae does not seem to include species that appear to relate it to more than one other group. In contrast to this, not only do the other Series within the Subsection Ciliatae tend to grade into one another, but the Subsections Ciliatae and Angustifoliae are scarcely discontinuous, members of the Series Alpinae in the Ciliatae approaching quite close to E. salisburgensis of the Angustifoliae.

The Series Hirtellae, it seems, ought both on morphological and cytological grounds to be raised to the rank of subsection, and the definition of the Subsection Ciliatae amended to exclude them.

Pugsley (1930) excluded the long-glandular forms known as E. brevipila var. notata and var. reayensis from the Hirtellae, and this proves to be justified by the cytology.

Hybridisation

Although diploid and tetraploid species were reported as long ago as 1932, there appears to have been no consideration of these counts in relation to the hybrids reported to occur. The existence of diploid and tetraploid species imposes a limitation on hybridisation and enables members of the two series to exist together, and remain distinct. In fact the results of diploid-tetraploid hybridisation appear to go beyond the formation of an occasional triploid, and it is intended to devote a future paper to this subject. Here it may be added that diploid and tetraploid species commonly grow together and that triploids are rare, that described above being the only one I have found in two seasons of active field work.

Origin of Tetraploids

The situation in the triploid E. anglica × micrantha indicates that homology exists between the set of chromosomes present in E. anglica and half the set in E. micrantha. E. micrantha must therefore be an allotetraploid. The simplest inference would be that E. anglica at some time crossed with a distantly related diploid, and that as a result of chromosome doubling in the offspring, E. micrantha arose. However, owing to the close relationship of the various tetraploids, as evidenced by their morphological similarity and the frequent occurrence of fertile hybrids among them, it is possible that an inference of this type may apply to the group of tetraploid species as a whole, and that E. micrantha is a subsequent descendant of the original tetraploid. Similarly E. anglica itself may not have been concerned in giving rise to tetraploids; a related present-day or ancestral species may have been responsible.

Another possibility is the repeated formation of tetraploid forms from different pairs of diploids from the same two groups.

It is probable that one should consider the origins of groups of species rather than of individual species. The question arises whether the other diploid group exists today, and if so, where. The only European groups not found in Britain are the Series Pectinatae and Alpinae; these fall within the Ciliatae and include species which form a series linking the Ciliatae with E. salisburgensis in the Angustifoliae. It is therefore unlikely that they include diploid species. Within the Angustifoliae, however, there is a sharp discontinuity between the salisburgensis complex, and the two closely related species E. cuspidata Host and E. tricuspidata L. A chromosome survey is needed, including these two species, and covering the other subsections of the Section Semicalcaratae, namely the Alpicoiae and Japonicae Pugsley (1936) of Japan, and also the Section Atlanticae Pugsley (l.c.) of the Azores. It is doubtful if the Alpicoiae and Japonicae are very distinct from one
THE CYTOLOGY OF BRITISH SPECIES OF **EUPHRASIA**

another. They possess rounded, cuneate-based leaves with rounded teeth, and the former includes both eglandular and long-glandular forms. The mere discovery of other diploid groups would not, of course, identify that which was concerned in giving rise to the European tetraploids.

**Endemism**

*Euphrasia* shows a high rate of endemism. Thus of the species counted, *E. foulaensis*, *E. marshallii*, *E. occidentalis*, *E. confusa*, *E. pseudokerneri*, *E. rivularis*, and *E. anglica* are endemic to the British Isles, or almost so. (*E. occidentalis* occurs in Brittany and probably in Belgium, and *E. confusa* and *E. foulaensis* occur in the Faeroes.) The cytological work shows that in these cases variation in chromosome number is not the cause of the multiplicity of species, many of which are very localized in distribution. Uniformity of chromosome number appears to be the rule, so that it seems improbable that any other British endemics owe their existence to the possession of different chromosome numbers.

**Summary**

Chromosome counts at pollen mother cell meiosis were made in fifteen British *Euphrasia* forms, covering thirteen species.

The method used is outlined and the selection of material for fixing described.

The numbers \( n = 22 \) and \( n = 11 \) were observed. All previously reported numbers are listed, and a table of all known numbers is presented (p. 105). In addition to the regular numbers, *E. brevipila* var. *notata* showed pairing failure in one pair of chromosomes, and plants were found with \( 2n = 44 + 1 \), presumably caused by this pairing failure; also a single triploid individual was observed, which most probably forms 11 bivalents and 11 univalents at metaphase. This plant was a hybrid between *E. micrantha* and *E. anglica*.

The relation of chromosome numbers to classification is discussed. The division between diploids and tetraploids coincides with one of the divisions of Pugsley's classification, the Series *Hirtellae* being diploid and other groups tetraploid. The raising of the *Hirtellae* to the rank of Subsection is recommended on morphological as well as cytological grounds. The anomalous forms *E. brevipila* var. *notata* and var. *reayensis* are tetraploids; this confirms their exclusion by Pugsley from the Series *Hirtellae*.

The existence of diploid and tetraploid series makes it possible for certain pairs of species to grow in company and remain distinct. This they frequently do; triploids, however, are very rare.

The cytology of the triploid shows that *E. micrantha* is an allotetraploid. In considering the origin of tetraploids, groups should be probably considered as a whole, since the species they comprise are closely related to one another. *E. anglica* is perhaps not a direct ancestor of a tetraploid, and *E. micrantha* is probably derived by divergence from a primitive tetraploid.

A chromosome survey of other groups of *Euphrasia*, particularly those within the Section *Semicalcaratae*, is needed.

Differences of chromosome number are not the cause of the multiplicity of *Euphrasia* species.

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Figs. 1 & 2. Meiosis in *Euphrasia anglica × micrantha* (185C), x 1000. Interpretative diagrams of two cells; fig. 2 corresponds with fig. 6. Univalents are shown solid, bivalents in outline. The arrows indicate bodies that look like multivalents (see text).

Fig. 3. Fig. 4.

Fig. 5. Fig. 6.

Figs. 3–6. Pollen mother cell meiosis in *Euphrasia*. Metaphase I, except fig. 5, x 1000. Fig. 3, *E. pseudokernerii* (E136). Fig. 4, *E. brevipila var. notata* (E254). Fig. 5, *E. hirtella* (E253), metaphase II. Fig. 6, *E. anglica × micrantha* (E185C).