THE CYTOLOGY OF BRITISH SPECIES OF *EUPHRASIA*

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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 Á. & D. Löve (1948) report that Sörensen and Westergaard found n=22 in *E. frigida* Pugsl., in Greenland. Maude (1939) records 2n=44 for "*E. confusa* Pugsl. (*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. pseudokerneri*, 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°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. scotica* 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* Pugsl. E311, Bettyhill, W. Sutherland, showed n=22.

(5) *E. curta* Fries var. *rupestris* Pugsl. Cultivated material (E100) showed n=22. E100 was collected in Cwm Idwal, Caernarvon, in June 1952, and consisted of herbarium specimens of *E. cambrica* Pugsl., and turves. The only plants that persisted in the turves were *E. curta* var. *rupestris*, 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. *rupestris* 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. Löhr. Two counts were obtained, both of n=22, one from E134, Bedford Purlieus, Northants., the other from E149, Banstead Downs, Surrey.

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

(9) *E. pseudokerneri* Pugsl. 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* Pugsl. 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* Pugsl. 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* Pugsl. 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* Pugsl. 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) Pugsl. 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 3·1 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. rostkoviana*, 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 of British species of Euphrasia *arranged according to Pugsley's classification, with* E. minima, *showing their haploid chromosome numbers.*

SECTION SEMICALCARATAE					
Subsection Ciliatae					
Series Latifoliae		Series Nemorosae		Series Brevipilae	
micrantha	22	*rotundifolia*		*borealis*	
scotica	22	*marshallii*	22	*brevipila*	22
rhumica		*curta* var. *rupestris*	22	var. *notata*	22
frigida	22*	*cambrica*		var. *reayensis*	22
foulaensis	22	*occidentalis*	22	Series Hirtellae	
eurycarpa		*nemorosa*	22	*rostkoviana*	11*
campbelliae		*heslop-harrisonii*		*montana*	11*
(*minima*)	22*	*confusa*	22	*rivularis*	11
		pseudokerneri	22	*anglica*	11
				hirtella	11
Subsection Angustifoliae					
E. salisburgensis	22*				

* 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 a as 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 *Alpicolae* and *Japonicae* Pugsley (1936) of Japan, and also the Section *Atlanticae* Pugsley (*l.c.*) of the Azores. It is doubtful if the *Alpicolae* and *Japonicae* are very distinct from one

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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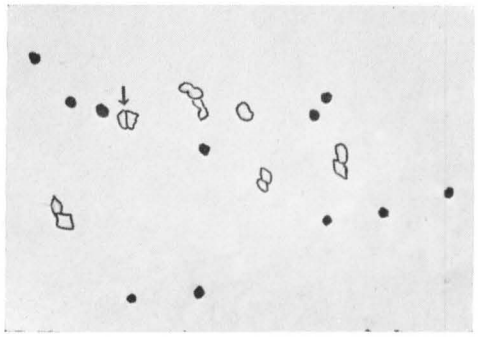


Fig. 1.

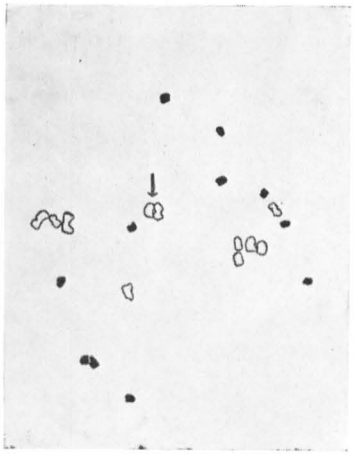
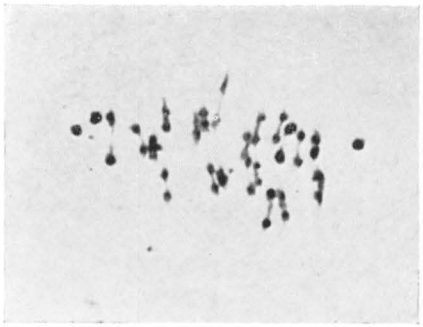


Fig. 2.

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.



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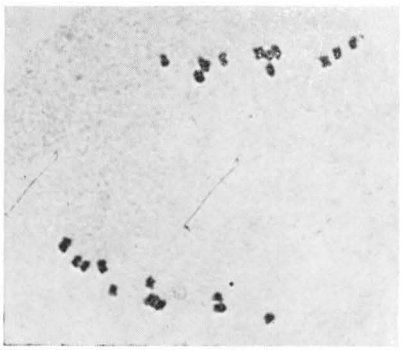

Fig. 5.

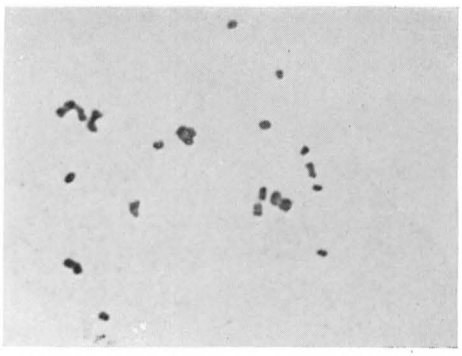

Fig. 6.

Figs. 3–6. Pollen mother cell meiosis in *Euphrasia*. Metaphase I, except fig. 5, x 1000.
Fig. 3. *E. pseudokerneri* (E136). Fig. 4, *E. brevipila* var. *notata* (E254).
Fig. 5, *E. hirtella* (E253), metaphase II. Fig. 6, *E. anglica* × *micrantha* (E185C).