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Artificial hybrids between some European diploid species of *Euphrasia*

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ABSTRACT

Euphrasia alpina (diploid) has been crossed with the diploids *E. rostkoviana* and *E. picta*, and morphologically intermediate hybrid progeny have been obtained. Pollen-mother-cell meiosis was slightly abnormal in the hybrid of *E. rostkoviana* and strongly so in the hybrid of *E. picta*. The results are discussed in relation to the origin of tetraploid species.

INTRODUCTION

It has been shown previously that the chromosome numbers of numerous European *Euphrasia* taxa are on two levels, diploid ($n = 11$) and tetraploid ($n = 22$) (Yeo 1954, 1970; Favarger 1969; Feoli & Cusma 1974). Yeo (1966) has further shown that on either ploidy level interspecific hybrids can be raised. On the tetraploid level enough crosses were made to show that the fertility of the hybrids is roughly proportional to the morphological similarity of the parents. It is now possible to report interspecific crosses on the diploid level additional to the only one previously made (which was between *E. anglica* Pugsl. and the form of *E. hirtella* Reut. found in Bretagne (Yeo 1966)).

Plants of two stocks of *E. alpina* Lam. (serial nos. E1072 and E1091) were pollinated in 1969 with pollen from *E. rostkoviana* Hayne subsp. *rostkoviana* (E1105) and *E. picta* Wimmer subsp. *picta* (E1113 and E1119A). Places of origin and available chromosome counts of these samples are given in the Appendix. The chromosome number of E1105 was not counted, but in *E. rostkoviana* subsp. *rostkoviana* from other localities it is $n = 11$ (Witsch 1932, Yeo 1970). The taxonomic problem surrounding the eglandular *E. picta* and the glandular-hairy *E. rostkoviana* has been briefly indicated elsewhere (Yeo 1970, 1972). Their recognition as separate species, as in *Flora Europaea* (Yeo 1972), is maintained here for the sake of convenience despite the evidence of intergradation presented by Schaeftlein (1967).

RESULTS

SUCCESS OF CROSSES

The seeds and plants resulting from the cross-pollinations are enumerated in Table 1. Some of the flowers pollinated were already open when emasculated, and one of those of E1091 pollinated

TABLE 1. RESULTS OF INTERSPECIFIC POLLINATIONS OF *EUPHRASIA ALPINA*

Female parent	No. of flowers pollinated	Pollen source		Apparently good seeds obtained	Progeny: Died young	Progeny: Hybrids	Progeny: Selfs
E. alpina							
E1091, plant 6	10	*E. rostkoviana*	E1105, plant 5	30	0	2	0
E1091, plant 8	5	*E. picta*	E1119A, plant 6	41	3 or 4	2	2
E1072	5	*E. picta*	E1113	11	0	0	0

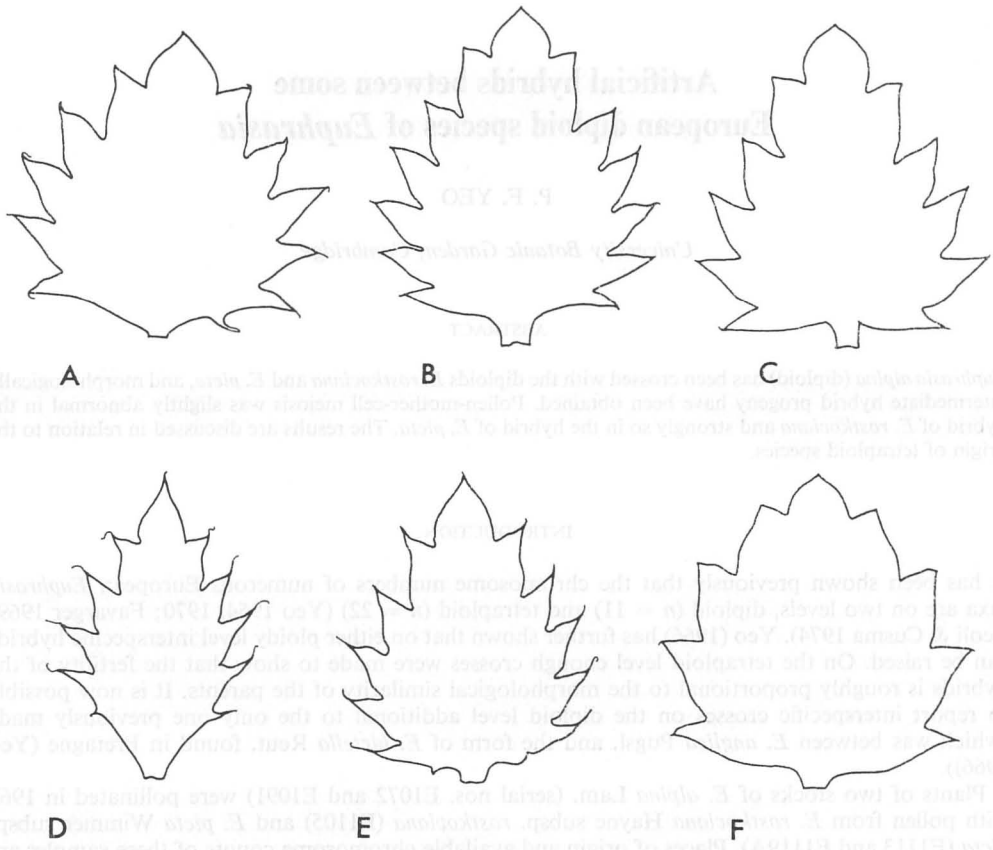

FIGURE 1. Leaves of *Euphrasia*. A. *E. alpina* E1091, plant 6, leaf 8. B. *E. alpina* E1091 × *E. rostkoviana* E1105, plant 1, leaf 7. C. *E. rostkoviana* E1105, plant 5, leaf 8. D. *E. alpina* E1091, plant 8, leaf 9. E. *E. alpina* E1091 × *E. picta* E1119A, plant 1, leaf 9. F. *E. picta* E1119A, plant 6, leaf 9. Leaves are numbered according to node, counted from base of plant, excluding cotyledonary node.

with E1119A was probably self-pollinated before it was crossed. All the seeds which appeared to be viable (except some from this last-mentioned flower) were imperfect in having obviously undersized contents and a normal-sized, but more or less crumpled, testa. Nevertheless, as Table 1 shows, some of them germinated. If it is assumed that 10 seeds per capsule is a normal production (*cf* Yeo 1966), the proportion of seeds with apparently significant swelling ranges from 22% to 80%.

In addition to the hybrids listed in Table 1, among plants of *E. alpina* (E1091) raised in 1970 from open-pollinated plants grown in 1969 there were two plants which were also apparently *E. alpina* × *E. rostkoviana* subsp. *rostkoviana*. The pollen parent of these must have been either E1105 or a sample from the Grossglocknerstrasse, Land Salzburg, Austria. The large and attractive flowers of *E. alpina* have a structure which ensures that most seed is produced as a result of insect-pollination; open-pollinated capsules of E1091 contained some normal seeds and some imperfect ones, and some of the latter doubtless had resulted from cross-pollination with other species.

MORPHOLOGY OF THE HYBRIDS

The leaf-shape and flower-shape of the hybrids and parents are illustrated in Figs. 1 and 2. Because climatic conditions were drier in 1970 than in 1969, the plants were less vigorous in that year and their corollas and leaves were smaller. However, in Fig. 1 the leaves have been unequally magnified to make them all approximately equal in length.

E. alpina (E1091) × *E. rostkoviana* subsp. *rostkoviana* (E1105)
The habit of the hybrids combines the short cauline internodes of E1105 with the rather stiff, ascending branches of E1091 (the branches of E1105 are flexuous).

The leaves of E1091, plant 6, are exceptionally broad for *E. alpina* (Fig. 1A). It is possible to see an intermediate condition in the hybrid in the shape of the leaf-base and the acuminate teeth of the leaf. The bristles terminating the teeth of leaves at higher nodes are fairly long in the hybrids, but inspection shows that on average they are shorter than those of E1091. The leaves also bear longer glandular hairs and denser eglandular hairs than in E1091, differences evidently due to the influence of the densely long-glandular *E. rostkoviana.*

Fig. 2A shows that the same plant of *E. alpina* also had broad corolla lobes. The hybrid is intermediate not only in the details of the lobing but also in the shape of the widened distal part of the tube. The corollas of E1091 were lilac and in E1105 they were white with a lilac upper lip. In the hybrids the corollas were almost white at first and rather strongly lilac finally, the degree of intensification being greater than is usual in lilac-flowered *Euphrasiae*. In *Euphrasia* corollas, any lilac colouring is usually weaker around the yellow spot on the lower lip. However, in both hybrids of this cross it retained its intensity and actually transgressed the boundary of the yellow spot, giving the latter a brownish border from the blending of the two pigments.

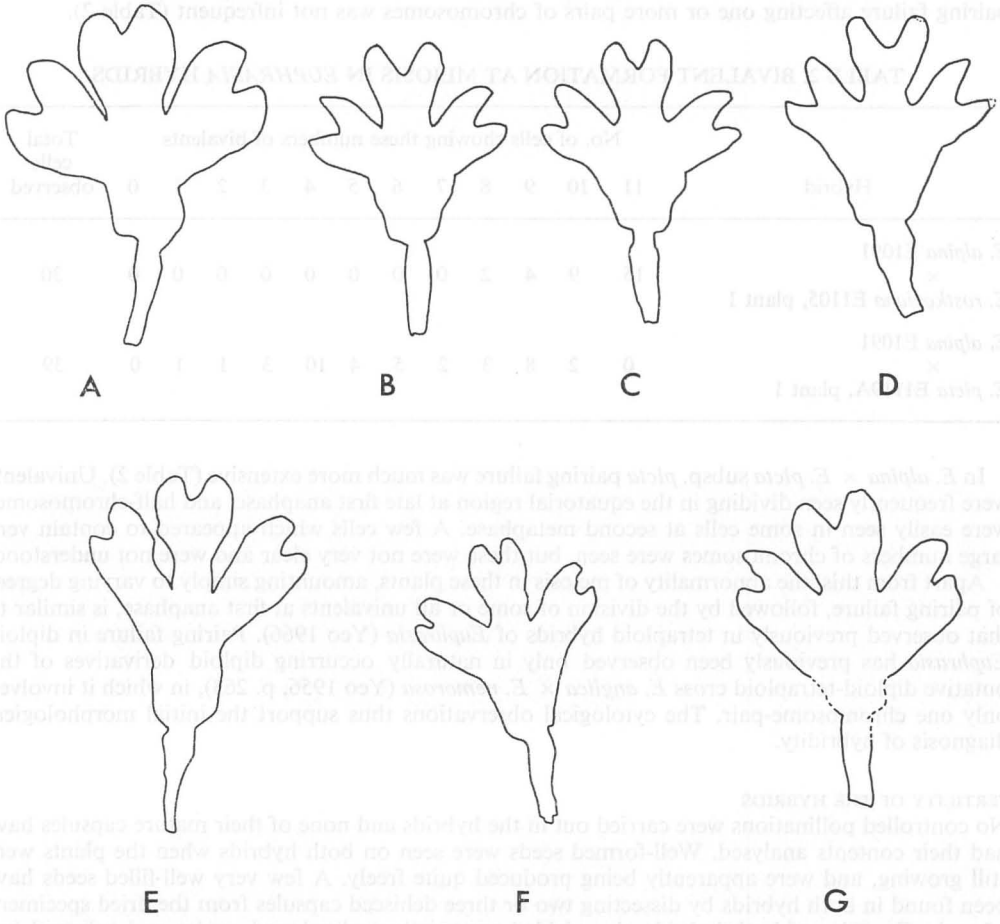

FIGURE 2. Corolla-lip and corolla-tube of *Euphrasia*. A. *E. alpina* E1091, plant 6. B. *E. alpina* E1091 × *E. rostkoviana* E1105, plant 1. C. The same, plant 2. D. *E. rostkoviana* E1105, plant 5. E. *E. alpina* E1091, plant 8. F. *E. alpina* E1091 × *E. picta* E1119A, plant 1. G. *E. picta* E1119A, plant 6.

E. alpina (E1091) × *E. picta* subsp. *picta* (E1119A)
The internodes of the hybrid are intermediate between the long ones of *E. alpina* and the short ones of *E. picta* subsp. *picta*. One plant of this cross was disordered throughout its life, the leaves and flowers being deformed. However, the habit and colouring are like those of its sibling and it is thought that it is a hybrid. Similar disorder has occasionally been seen in non-hybrid *Euphrasia* plants in cultivation, and it cannot therefore be attributed to partial hybrid incompatibility in this case.

The narrow leaves of E1091, plant 8 (Fig. 1D), are more typical of *E. alpina* than those of plant 6, and the intermediate condition of the hybrid leaf is easily appreciated, owing to the great difference between the parents.

Owing to the similarity of the parental corollas that of the hybrid is not very distinct. It does, however, show the same shape in the widened part of the tube as in *E. alpina* × *E. rostkoviana*.

MEIOSIS IN THE HYBRIDS

The stages of meiosis observed in *E. alpina* × *E. rostkoviana* subsp. *rostkoviana* were first and second metaphase, first anaphase (early and late) and first telophase. In second metaphase a chromosome was occasionally seen away from the equator; these were probably derived from chromosomes which had failed to pair in first prophase, but had divided at first anaphase. In the first division, pairing failure affecting one or more pairs of chromosomes was not infrequent (Table 2).

TABLE 2. BIVALENT FORMATION AT MEIOSIS IN *EUPHRASIA* HYBRIDS

Hybrid	No. of cells showing these numbers of bivalents												Total cells observed
	11	10	9	8	7	6	5	4	3	2	1	0	
E. alpina E1091 × *E. rostkoviana* E1105, plant 1	15	9	4	2	0	0	0	0	0	0	0	0	30
E. alpina E1091 × *E. picta* E1119A, plant 1	0	2	8	3	2	5	4	10	3	1	1	0	39

In *E. alpina* × *E. picta* subsp. *picta* pairing failure was much more extensive (Table 2). Univalents were frequently seen dividing in the equatorial region at late first anaphase, and half-chromosomes were easily seen in some cells at second metaphase. A few cells which appeared to contain very large numbers of chromosomes were seen, but these were not very clear and were not understood.

Apart from this, the abnormality of meiosis in these plants, amounting simply to varying degrees of pairing failure, followed by the division of some or all univalents at first anaphase, is similar to that observed previously in tetraploid hybrids of *Euphrasia* (Yeo 1966). Pairing failure in diploid *Euphrasia* has previously been observed only in naturally occurring diploid derivatives of the putative diploid-tetraploid cross *E. anglica* × *E. nemorosa* (Yeo 1956, p. 263), in which it involved only one chromosome-pair. The cytological observations thus support the initial morphological diagnosis of hybridity.

FERTILITY OF THE HYBRIDS

No controlled pollinations were carried out in the hybrids and none of their mature capsules have had their contents analysed. Well-formed seeds were seen on both hybrids when the plants were still growing, and were apparently being produced quite freely. A few very well-filled seeds have been found in both hybrids by dissecting two or three dehisced capsules from the dried specimens of each. Capsules of both hybrids also yielded apparently undeveloped ovules and enlarged but empty or nearly empty testas, but these could also be found in the open-pollinated capsules of *E. alpina*.

DISCUSSION

There is a close morphological similarity between *E. picta* subsp. *picta* and at least some plants of *E. rostkoviana* and, as already mentioned, they sometimes intergrade. *E. alpina*, on the other hand, is sufficiently distinct morphologically to be classified apart from the other two species. Accordingly, when I published chromosome counts for *E. picta* and *E. alpina* (Yeo 1970), I suggested that *E. alpina* might have combined with one of them to give rise, by amphidiploidy, to some or all of the European tetraploids outside the section *Augustifoliae*. Morphologically the artificial hybrids are not dissimilar to species such as *E. stricta* Lehm., *E. arctica* Lange ex Rostrup and *E. nemorosa* (Pers.) Wallr.

The present results, showing successful crossing of *E. alpina* with the other two species, and the occurrence of varying degrees of chromosomal non-homology in the hybrid, are grounds for suggesting that my hypothesis might be followed up by someone more suitably placed to do it than I.

However, it seems that the hybrids reported here are not so sterile that they might not occur naturally as populations of morphologically intermediate plants with the diploid chromosome number. In fact I have found a population of modified *E. alpina* which might have resulted from crossing with *E. rostkoviana* subsp. *rostkoviana* (which accompanied it) in Switzerland (E1107A & B, see Appendix). The larger plants of this gathering are similar to the Pyrenean *E. asturica* Pugsl., which Pugsley (1932) placed in Series *Nemorosae* and which I previously listed as a synonym of *E. stricta* (Yeo 1972).

REFERENCES

FAVARGER, C. (1969). Notes de caryologie alpine, 5. *Bull. Soc. neuchâtel. Sci. nat.*, **92**: 13–30.

FEOLI, E. & CUSMA, T. (1974). Sulla posizione sistematica de *Euphrasia marchesettii* Wettst. *Giorn. bot. Ital.*, **108**: 145–154.

PUGSLEY, H. W. (1932). Notes on *Euphrasia*. *J. Bot.* (*Lond.*), **70**: 200–204.

SCHAEFTLEIN, H. (1967). Beiträge zur Kenntnis einiger mitteleuropäischer Euphrasien, 1. Der taxonomische Wert der drüsigen Behaarung. *Phyton* (*Horn*), **12**: 48–90.

WITSCH, H. VON (1932). Chromosomenstudien am mitteleuropäischen Rhinantheen. *Österr. bot. Z.*, **81**: 108–141.

YEO, P. F. (1954). The cytology of British species of *Euphrasia*. *Watsonia*, **3**: 101–108.

YEO, P. F. (1956). Hybridisation between diploid and tetraploid species of *Euphrasia*. *Watsonia*, **3**: 253–269.

YEO, P. F. (1966). The breeding relationships of some European *Euphrasiae*. *Watsonia*, **6**: 216–245.

YEO, P. F. (1970). New chromosome counts in *Euphrasia*. *Candollea*, **25**: 21–24.

YEO, P. F. (1972). *Euphrasia*, in TUTIN, T. G. *et al.*, eds. *Flora Europaea*, **3**: 257–266. Cambridge.

APPENDIX

SOURCES OF MATERIAL AND LOCATION OF VOUCHERS

Specimens of the hybrids and their parents are in **herb. P. F. Yeo** and of the latter in **CGE.**

E. alpina

E1072. France: Alpes Maritimes. Grown at Cambridge in 1968 and 1969 from seed supplied by the Muséum National d'Histoire Naturelle, Paris, 1968. $n = 11$ (Yeo 1970).

E1091. Switzerland: Above Brunnen, Simplon Pass, Valais, 1380m, collected 1968. $n = 11$ (Yeo 1970).

E. alpina ? × *E. rostkoviana* subsp. *rostkoviana*

E1107A & B. Switzerland: Blatten, N. side of Rhône Valley near Brig, Valais, *c* 1350m, collected 1968.

E. rostkoviana subsp. *rostkoviana*

E1105. Switzerland: Near Bitsch, 3km N.E. of Brig, Valais, 700m, collected 1968.

E. picta subsp. *picta*

E1113. Austria: Hochmais, Grossglocknerstrasse, Land Salzburg, 1850m, collected 1968.

E1119A. Austria: Enzinger Boden, Stubbachtal, Land Salzburg, 1480m, collected 1968. $n = 11$ (Yeo 1970).

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