

Studies on variation and evolution in *Centaureum erythraea* Rafn and *C. littorale* (D. Turner) Gilmour in the British Isles

2. Cytology

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ABSTRACT

Chromosome counts of *Centaureum erythraea* Rafn and *C. littorale* (D. Turner) Gilmour from northern Europe are given. They confirm those made by Zeltner (1970) and show both species to be tetraploid ($2n = 40$) with regular meiosis.

Morphologically intermediate (F_1 -like) plants from Ainsdale, S. Lancs. (v.c. 59), were found to be tetraploid with irregular meiosis. They were almost sterile, as were F_1 -like plants from Newborough, Anglesey (v.c. 52), and were almost identical in their morphology and cytology to artificial F_1 hybrids.

Morphologically intermediate plants from northern Germany were found to be tetraploid, but with regular meiosis and a high fertility. It is suggested that they are stabilized derivatives of the F_1 hybrid.

Hybrid plants from St Annes, W. Lancs. (v.c. 60), which resemble *C. erythraea* in their morphology and are probably backcrosses to this species, were found to be tetraploid with regular meiosis and a high fertility.

Hybrid plants from Ainsdale, Freshfield and Hightown, S. Lancs. (v.c. 59), which closely resemble *C. littorale* in their morphology, were found to be hexaploid ($2n = 60$) with regular meiosis and a high fertility. They are shown to breed true, to be isolated from their tetraploid parents and other (tetraploid) hybrids by a difference in chromosome number, and to be able to compete successfully with their parents. They are, therefore, considered to constitute a new species, and a possible origin involving hybridization and polyploidy is discussed.

INTRODUCTION

Prior to Zeltner's work (1961, 1962, 1963, 1966, 1967) only four species of the genus *Centaureum* Hill had been examined cytologically, and the results obtained by different authors contradicted each other so much that it was impossible even to establish a basic number for the genus (Wulff 1937, Warburg 1939, Rork 1949, Mesquita Rodrigues 1953, Khoshoo & Khushu 1966, Brink 1967).

Zeltner made numerous, reliable chromosome counts of all the European species and finally produced a comprehensive treatment summarizing his work on the genus (Zeltner 1970). He found all the karyotypes to be very similar and decided that they were of no taxonomic value. He also established two basic chromosome numbers for the European species, of $x = 11$ for *C. spicatum* (L.) Fritsch and $x = 10$ for all the other species. Six of the latter were found to be diploid ($2n = 20$), two tetraploid ($2n = 40$) and the other three species were found to have both diploid and tetraploid races, the diploids occurring in the Mediterranean region and the tetraploids in central and northern Europe. His work showed the importance of polyploidy, and of the resulting genetical isolation, in the evolution of the genus.

CENTAUREUM ERYTHRAEA RAFN

The first chromosome count ($2n = 42$) for this species was determined by Rork (1949) on a plant from New York. Zeltner (1962) carried out chromosome counts on 113 populations from Europe.

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Of these, 16 from southern Europe were found to be diploid ($2n = 20$) with regular meiosis, while the other 97, all from northern Europe, were found to be tetraploid ($2n = 40$) with regular meiosis. It is not known how the tetraploids arose, but Zeltner suggested that it could have been by autopolyploidy.

C. capitatum (Willd.) Borbás, which is now considered to be only a variety of *C. erythraea* (Melderis 1972, Ubsdell 1976), was also found by Zeltner to be tetraploid with regular meiosis.

CENTAURIUM LITTORALE (D. TURNER) GILMOUR

The first chromosome count ($n = 19$) for this species was determined by Wulff (1937) on material from northern Germany. Warburg (1939) recorded $2n = 38$ and $2n = 56$ on material from the British Isles, while Brink (1967) reported that most plants of this species from the Netherlands had $2n = 40$ although she had found a few plants with $2n = 38$ or $2n = 42$. Zeltner (1962) examined 15 populations from Europe and similarly found all to be tetraploid with regular meiosis. The diploid relatives of this species are confined to the Iberian peninsula and southern France, and Zeltner suggested that *C. littorale* could have been derived from them either by autopolyploidy or by allopolyploidy.

NATURALLY OCCURRING HYBRIDS

Zeltner (1970) examined some hybrid plants (*C. erythraea* \times *C. littorale*) from Ainsdale, S. Lancs. (v.c. 59). He found 40 chromosomes at mitosis, but observations of meiosis showed some of the cells dividing regularly to give two equal groups of 20 chromosomes, and others showing irregular division with groups of 20/24 and 16/24. 80% of the pollen was distorted and sterile.

K. Jakobsen (in litt. 1972) reported that tetraploid hybrids between these two species occur on the coasts of Denmark. Some of the hybrids were almost sterile with irregular meiosis, while others appeared to be cytologically stable.

This paper is concerned with the cytology and pollen fertility of plants of *C. erythraea* and *C. littorale* from northern Europe (chiefly from the British Isles), their naturally occurring hybrids (Ubsdell 1976) and artificial hybrids.

METHODS

CHROMOSOME COUNTS

Somatic chromosomes were examined at mitosis in the root-tips. Initially they were difficult to count as all the chromosomes tended to conglomerate on the metaphase plate. Eventually, however, pre-treatment with saturated, aqueous paradichlorobenzene for twelve hours before fixation in 3:1 ethanol:acetic acid was found to give satisfactory preparations with the chromosomes well spread. After fixation the root-tips were softened in N HCl for three minutes before washing and staining in propionic-orcein.

Buds were fixed in 3:1 ethanol:acetic acid and stored in 70% ethanol. After softening in 1:1 ethanol:concentrated HCl, good results were obtained for the meiotic chromosomes by staining in propionic-orcein; acetic-orcein and acetocarmine gave poor results.

All preparations were made permanent by freezing with Arcton 12, dehydrating in ethanol and mounting in Euparal.

POLLEN FERTILITY

Fresh pollen grains were mounted in cotton blue/lactophenol. Spherical pollen grains took up the stain and were considered to be viable, while unstained, distorted, elliptical grains were considered to be infertile. 100 pollen grains were scored for each plant.

RESULTS

Details of the chromosome number, pairing at meiosis and pollen fertility are given for various collections of *C. erythraea* (Table 1), *C. littorale* (Table 2), and their natural (Table 3) and artificial (Table 4) hybrids.

TABLE 1. CHROMOSOME NUMBER, MEIOTIC PAIRING AND POLLEN FERTILITY OF *C. ERYTHRAEA*

Single species populations	Meiosis		Root-tip mitosis	Pollen fertility
	metaphase I (No. of bivalents)	anaphase I		
4. Orpington, W. Kent, v.c. 16	20	2n = 40	—	84%
5. Folkestone, E. Kent, v.c. 15	20	—	—	93%
6. Luccombe, Isle of Wight, v.c. 10	20	2n = 40	—	92%
7. Sandown, Isle of Wight, v.c. 10	20	—	—	90%
8. Swanage, Dorset, v.c. 9	20	2n = 40	—	89%
10. Bonchurch, Isle of Wight, v.c. 10	20	—	—	89%
11. Freshwater, Isle of Wight, v.c. 10	20	2n = 40	—	90%
12. Steyning, W. Sussex, v.c. 13	20	—	—	96%
23. Aberlady, Haddington, v.c. 82	—	—	2n = 40	92%
25. Newbiggin, S. Northumberland, v.c. 67	—	—	2n = 40	87%
27. Fanore, Clare, v.c. H9	20	2n = 40	2n = 40	86%
28. Funshin, S.E. Galway, v.c. H15	—	—	2n = 40	91%
J. Minsmere, E. Suffolk, v.c. 25	20	—	2n = 40	90%
L. Minsmere, E. Suffolk, v.c. 25	20	—	—	90%
Mixed populations				
14. Hightown, S. Lancs., v.c. 59	20	—	—	82%
15. Freshfield, S. Lancs., v.c. 59	20	—	2n = 40	80%
20. St Annes, W. Lancs., v.c. 60	20	2n = 40	2n = 40	88%
21. Ainsdale, S. Lancs., v.c. 59	20	2n = 40	2n = 40	96%
AN. Newborough, Anglesey, v.c. 52	20	—	—	86%
Continental plants raised in greenhouse (all seed obtained from Botanic Gardens)				
Rügen, Germany	20	—	—	90%
Wageningen, Netherlands	—	—	2n = 40	92%
Copenhagen, Denmark	—	—	2n = 40	90%

TABLE 2. CHROMOSOME NUMBER, MEIOTIC PAIRING AND POLLEN FERTILITY OF *C. LITTORALE*

Single species populations	Meiosis		Root-tip mitosis	Pollen fertility
	metaphase I (No. of bivalents)	anaphase I		
D. Ainsdale, S. Lancs., v.c. 59	20	2n = 40	—	94%
G. Ainsdale, S. Lancs., v.c. 59	20	2n = 40	2n = 40	88%
H. Ainsdale, S. Lancs., v.c. 59	20	2n = 40	2n = 40	89%
BA. Newborough, Anglesey, v.c. 52	20	2n = 40	—	79%
N. Newborough, Anglesey, v.c. 52	20	2n = 40	—	86%
24. Holy Island, Cheviot, v.c. 68	20	2n = 40	2n = 40	84%
22. Nairn, v.c. 96b	20	2n = 40	2n = 40	92%
Mixed populations				
E. Ainsdale, S. Lancs., v.c. 59	20	2n = 40	2n = 40	86%
21. Ainsdale, S. Lancs., v.c. 59	20	2n = 40	2n = 40	88%
14. Hightown, S. Lancs., v.c. 59	—	—	2n = 40	88%
16. Freshfield, S. Lancs., v.c. 59	20	2n = 40	2n = 40	90%
20. St Annes, W. Lancs., v.c. 60	20	—	—	88%
AN. Newborough, Anglesey, v.c. 52	20	2n = 40	—	80%
Continental plants raised in the greenhouse (all seed obtained from Botanic Gardens)				
Rügen, Germany	20	2n = 40	—	88%
Blidö, Sweden	20	2n = 40	—	89%
Helsinki, Finland	—	—	2n = 40	88%
Copenhagen, Denmark	—	—	2n = 40	90%

TABLE 3. CHROMOSOME NUMBER, MEIOTIC PAIRING AND POLLEN FERTILITY OF NATURAL HYBRIDS

Cultivation code and status	Meiosis		Root-tip mitosis	Pollen fertility
	metaphase I (No. of bivalents, univalents and quadrivalents)	anaphase I (pattern of segregation and diploid no.)		
14. Hightown, S. Lancs.	30 II	30-30 ($2n = 60$) 31-26 ($2n = 57$) 29-28 ($2n = 57$) 31-25 ($2n = 56$) 26-25 ($2n = 51$) 26-24 ($2n = 50$)	—	45, 58, 63, 69, 70, 80, 81, 84%
Progeny in cultivation R60	30 II	30-30 ($2n = 60$)	$2n = 60$	81-90%
16. Freshfield, S. Lancs.	30 II	28-28 ($2n = 56$) 27-27 ($2n = 54$) 28-27 ($2n = 55$) 29-29 ($2n = 58$) 27-25 ($2n = 52$) 30-29 ($2n = 59$) and those given for 14	—	76, 82, 84, 88, 92, 94, 96%
Progeny in cultivation R61	30 II	30-30 ($2n = 60$)	$2n = 60$	85-90%
20. St Annes, W. Lancs.	20 II	20-20 ($2n = 40$)	—	88%
21. Ainsdale, S. Lancs.	—	$2n = 40$	—	0-10%
	30 II	$2n = 60$	—	80-90%
B. Ainsdale, S. Lancs.	—	—	—	80-88%
Progeny in cultivation R10	30 II	30-30 ($2n = 60$)	$2n = 60$	74-88%
R11	29/30 II	29-29 ($2n = 58$) 30-30 ($2n = 60$)	$2n = 60$	81-82%
R31	30 II	30-30 ($2n = 60$)	$2n = 60$	78-89%
R51	30 II	30-30 ($2n = 60$)	$2n = 60$	80-87%
R52	30 II	30-30 ($2n = 60$)	$2n = 60$	81-87%
Selfed progeny of R10, R11	30 II	30-30 ($2n = 60$)	$2n = 60$	80-90%
C. Ainsdale, S. Lancs.	—	$2n = 40$	—	0-10%
	—	—	—	67-80%
Progeny in cultivation R23	20 II 19 II+2 I 17 II+6 I	$2n = 40$	$2n = 40$	0-10%
R50	29 II 28 II+1 IV 26 II+4 I	31-29 ($2n = 60$) 31-27 ($2n = 58$) 30-25 ($2n = 55$) 30-24 ($2n = 54$)	$2n = 60$	67-87%
E. Ainsdale, S. Lancs.	—	$2n = 40$	—	0-10%
Progeny in cultivation R28	18 II+4 I 18 II+1 IV 15 II+1 IV+6 I 14 II+1 IV+8 I 13 II+1 IV+6 I and as for R23	$2n = 40$	$2n = 40$	0-10%
AN. Newborough, Anglesey	—	—	—	0-10%
FAL. Falshöft, Germany	20 II	20-20 ($2n = 40$)	—	77-85%
S.P. St Peter, Germany	20 II	20-20 ($2n = 40$)	—	78-82%

TABLE 4. CHROMOSOME NUMBER, MEIOTIC PAIRING, POLLEN FERTILITY AND SEED SET OF ARTIFICIAL HYBRIDS

Cultivation code and status	metaphase I	Meiosis anaphase I	Root-tip mitosis	Pollen fertility	\bar{x} seed set per capsule by open pollination
F ₁ 1	20 II	24-20 ($2n = 44$)	$2n = 40$	0-10%	0-6
2	19 II+2 I	23-20 ($2n = 43$)			
	18 II+4 I	22-19 ($2n = 41$)			
3	17 II+6 I	20-20 ($2n = 40$)			
	18 II+1 IV	20-18 ($2n = 38$)			
4	17 II+1 IV+2 I	19-19 ($2n = 38$)			
	16 II+1 IV+4 I	19-17 ($2n = 36$)			
	15 II+2 IV+2 I	20-15 ($2n = 35$)			
	14 II+2 IV+4 I				
	15 II+chs. of 5+5 I				
Tetraploid hybrid R23 \times <i>C. erythraea</i>					
5	20 II	20-20 ($2n = 40$)	$2n = 40$	67%	110
	18 II+1 IV				
	15 II+2 IV+2 I				
Tetraploid hybrid R23 \times <i>C. littorale</i>					
6	Not clear but IIs, IIIs and IVs	20-1-1-17 ($2n = 40$) 18-2-2-18	$2n = 40$	6-9%	2
Hexaploid hybrid \times <i>C. erythraea</i>					
7	24 II+2 I	28-22 ($2n = 50$)	$2n = 50$	26-54%	50
8	22 II+2 III	26-24 ($2n = 50$)			minute
(reciprocal crosses)	19 II+chs. of 4, 6	25-5-20 ($2n = 50$) 26-22+1 II ($2n = 50$) 21-21+4 II ($2n = 50$)			malformed
Hexaploid hybrid \times <i>C. littorale</i>					
9	20 II+chs. of 3, 3, 4	25-25 ($2n = 50$)	$2n = 50$	3-25%	10
10	20 II+chs. of 4, 4+2 I	25-1-24 ($2n = 50$)			minute
(reciprocal crosses)	19 II+chs. of 4, 6+2 I	25-2-23 ($2n = 50$)			malformed
	19 II+chs. of 6+6 I	24-8-18 ($2n = 50$)			
	18 II+chs. of 4, 6+2 I	20-15-15 ($2n = 50$)			
	18 II+rgs. of 4, 4+4 I	20-3-27 ($2n = 50$)			
	17 II+chs. of 3, 3, 4, 6	20-30 ($2n = 50$)			
	17 II+chs. of 3, 3, 4, 4+2 I				
	16 II+chs. of 4, 4, 4, 6				
	16 II+chs. of 3, 4, 6+4 I				
	16 II+rgs. of 4, 4+4 I				
	16 II+chs. of 3, 3, 5, 5				
Hexaploid hybrid \times Hexaploid hybrid					
11	30 II	30-30 ($2n = 60$)	$2n = 60$	79-89%	200

CENTAURIUM ERYTHRAEA

All plants of this species, both from single species populations and from mixed ones, were found to be tetraploid ($2n = 40$) with regular meiosis. All plants were highly fertile, with a pollen fertility of over 80% in single species and mixed populations (Table 1).

CENTAURIUM LITTORALE

All plants of this species, both from single species populations and from mixed ones, were also found to be tetraploid ($2n = 40$) with regular meiosis. All plants were highly fertile, with a pollen fertility of over 79% in single species and mixed populations (Table 2).

NATURALLY OCCURRING HYBRIDS

These hybrids showed a variety of chromosome numbers and meiotic irregularities, and seemed to fall into three main cytological types.

1. This is represented by the morphologically intermediate, F_1 -like plants from Ainsdale, S. Lancs. (Table 3: populations C, E & 21). These plants were tetraploid ($2n = 40$) with irregular meiosis. No clear metaphase counts were made, but preparations of first anaphase showed lagging chromosomes, fragments and apparent chromatid bridges. All plants had a very low pollen fertility of 0–10%.

Seed collected from these hybrids in the field was sown in the greenhouse and the progeny raised to maturity. These cultivated plants, which were at least second generation hybrids (Table 3: R23 & R28), were also found to be tetraploid ($2n = 40$) and to have irregular meiosis. The number of bivalents varied from 13 to 20, the other chromosomes forming univalents or occasionally a quadrivalent (Fig. 1: a & b). There were lagging chromosomes and other irregularities at first anaphase, and despite the large number of bivalents all plants had a low pollen fertility (0–10%).

Morphologically intermediate, F_1 -like plants from Newborough, Anglesey (v.c. 52) (Table 3: population AN), also had a low pollen fertility, but unfortunately no chromosome counts were made.

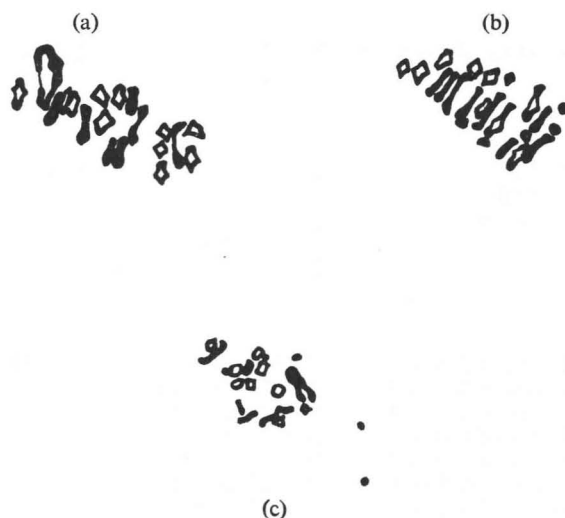


FIGURE 1. Tetraploid hybrids.

Progeny of morphologically intermediate, F_1 -like hybrids from Lancashire (a) R28 metaphase I of meiosis (18 II + 1 IV), (b) R23 metaphase I of meiosis (17 II + 6 I).

Artificial F_1 hybrid (c) metaphase I of meiosis (15 II + 5 I and a chain of 5).

2. This is represented by plants from St Annes, W. Lancs. (v.c. 60). Table 3, population 20), which closely resemble *C. erythraea* in their morphology and may be backcrosses to this species, and also by the morphologically intermediate plants from the northern German populations (Table 3: populations FAL. & S.P.). These plants were found to be tetraploid ($2n = 40$) with regular meiosis, and all were highly fertile with a pollen fertility comparable to that of the parents. Unfortunately, no progeny were raised in the greenhouse.

3. This is represented by the majority of hybrid plants from Ainsdale, Freshfield and Hightown, S. Lancs. (Table 3: populations 14, 16 & 21), which closely resemble *C. littorale* in their morphology and may possibly be backcrosses to this species. These plants were found to be basically

hexaploid ($2n = 60$), but meiotic counts of 50, 51, 52, 54, 55, 56, 57, 58 and 59 were also made, more than one number often being counted from the same individual. Three preparations of first metaphase showed complete pairing with 30 bivalents, but many other preparations were not clear enough for a count to be made. Twenty preparations of first anaphase showed cells dividing regularly into two equal groups of from 27 to 30 chromosomes, while a further ten preparations showed cells dividing irregularly into groups of 24/26, 25/26, 25/31, 27/28, 26/31, 28/29 chromosomes (Fig. 2). These plants showed a wide range of pollen fertility (45–96%), but the majority were quite fertile (over 80%).



FIGURE 2. Hexaploid hybrids (a) anaphase I of meiosis ($2n = 50$), (b) anaphase I of meiosis (31–26, $2n = 57$), (c) anaphase I of meiosis (30–30, $2n = 60$).

Wild-collected seed from these hybrids was sown in the greenhouse and the progeny raised to maturity. All plants were found to be basically hexaploid (counts of $2n = 60$ for root-tip chromosomes). A few plants (Table 3: R50) showed irregular meiosis, the number of bivalents varying from 26 to 29 with the other chromosomes forming univalents or occasionally a quadrivalent. Anaphase segregation was unequal, and the pollen fertility of these plants fell within the range 67–87%. However, the majority of plants (Table 3: R10, R11, R31, R51, R52, R60, R61) showed regular meiosis with complete pairing (30 bivalents) and equal anaphase segregation into two groups of 30 chromosomes. The pollen fertility of these cytologically stable, hexaploid plants was high (74–90%) and comparable to that of the parents.

Seed set by selfing these hexaploid hybrids in the greenhouse produced progeny identical to the parents in their morphology, cytology and fertility (Fig. 3, R10).

ARTIFICIAL F_1 HYBRIDS AND THEIR PROGENY

These hybrids resulted from crosses made in the greenhouse between *C. erythraea* and *C. littorale*. All were found to be morphologically intermediate between the parents (Fig. 3: 1–4), and tetraploid ($2n = 40$) with irregular meiosis (Table 4: 1–4). The number of bivalents varied from 14 to 20, the other chromosomes forming univalents and multivalents (Fig. 1c). There were also lagging chromosomes and other irregularities at first anaphase. Despite the large number of bivalents, pollen fertility was low (0–10%) and all plants set very little seed by artificial self-pollination.

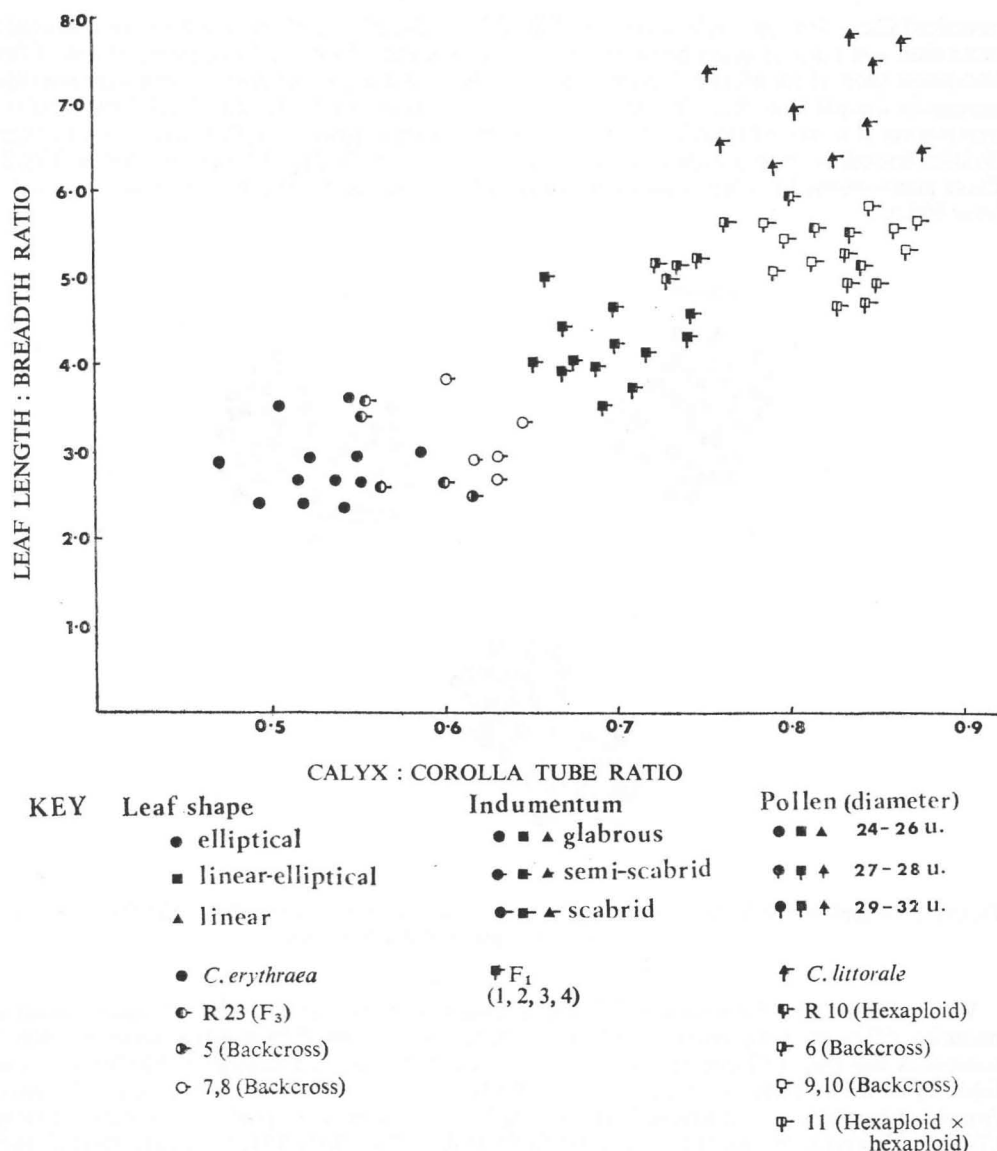


FIGURE 3. Scatter diagram comparing all artificial hybrids with plants of *C. erythraea*, *C. littorale* and their natural hybrids raised in the same greenhouse in the same year.

OTHER ARTIFICIAL HYBRIDS

Crosses between natural, almost sterile, tetraploid hybrids (R23) and natural, hexaploid hybrids produced pentaploid ($2n = 50$) progeny with irregular meiosis and a very low pollen fertility and seed set. No such plants were found in the wild.

Using the same natural, tetraploid hybrid (R23), backcrosses were made to *C. erythraea* and *C. littorale*. Surprisingly the two backcrosses had very different fertility. That to *C. littorale* was morphologically intermediate between the tetraploid and hexaploid hybrids, had irregular meiosis, a low pollen fertility and seed-set (Table 4: 6), and was unlike any plant found in the wild. By contrast, the backcrosses to *C. erythraea* closely resembled that species and had a more regular meiosis, higher pollen fertility and better seed set than both the natural and artificial F₁ hybrids

(Table 4: 5). These plants strongly resembled the fertile, tetraploid hybrids (Table 3: population 20) from St Annes, W. Lancs. in their morphology, cytology and fertility.

Backcrossing the natural, hexaploid hybrids (R10, R11) to *C. erythraea* produced progeny intermediate in morphology between the F_1 hybrid and *C. erythraea* (Fig. 3: 7 & 8), but the backcrosses to *C. littorale* closely resembled the hexaploid parents (Fig. 3: 9 & 10). Both backcrosses were pentaploid ($2n = 50$) with irregular meiosis, a low pollen fertility and poor seed-set (Table 4: 7-10). No plants with these characteristics were found in the wild.

DISCUSSION

Chromosome counts of *C. erythraea* and *C. littorale* from northern Europe show both species to be tetraploid ($2n = 40$) with regular meiosis. These results confirm those of Zeltner (1970) and Brink (1967), but contradict those of Wulff (1937), Warburg (1939) and Rork (1949). It is possible that faulty observation of this cytologically difficult material might explain the counts of $2n = 42$ for *C. erythraea* made by Rork (1949), and $2n = 38$ for *C. littorale* made by Wulff (1937) and Warburg (1939). It also seems probable that the count of $2n = 56$ for *C. littorale* by Warburg (1939) was made from the hexaploid hybrid between this species and *C. erythraea*, since the hexaploid hybrids closely resemble *C. littorale* and are easily mistaken for this species.

The artificial F_1 hybrids are shown to be tetraploid ($2n = 40$) with fairly good pairing at first metaphase, but irregularities at first anaphase and a low pollen fertility. It therefore seems likely that the morphologically intermediate (Hybrid-index 25-40 (Ubsdell 1976)), tetraploid plants from Ainsdale, S. Lancs., and Newborough, Anglesey, with irregular meiosis and low pollen fertility also belong to the F_1 generation, as do those examined by Zeltner (1970).

The morphologically intermediate, tetraploid plants (Hybrid-index 25-40) from northern Germany with regular meiosis and a high pollen fertility seem unlikely to belong to the F_1 generation, since artificial F_1 hybrids produced by crossing plants of *C. erythraea* and *C. littorale* from northern Germany were found to have irregular meiosis and a low pollen fertility similar to those produced by crossing British plants. It is possible that they are segregates of the F_1 hybrid which have become cytologically stable, and a similar suggestion was made by Jakobsen (*in litt.* 1972) to explain the origin of cytologically stable, fertile, tetraploid hybrids found in populations on the coast of Denmark.

The highly fertile, hexaploid, hybrid plants from Ainsdale, Freshfield and Hightown, S. Lancs. (Hybrid-index 45-50) could have been formed by one of several methods.

While it is possible that it may have been derived from an octoploid parent and a normal, tetraploid parent, this seems very unlikely since the number $2n = 80$ has never been recorded in *Centaureum*. It seems more likely that the hexaploid was produced from two tetraploid parents, *C. littorale* and *C. erythraea*, and this must almost certainly have involved an unreduced gamete, possibly as a result of non-disjunction and restitution during first meiotic division.

It is possible that the unreduced gamete was from *C. littorale* since the hexaploids most closely resemble this species. With three sets of chromosomes, two of which would be from *C. littorale*, they would also have at least 20 bivalents and it is possible that some of the *C. erythraea* chromosomes might also pair. Those that do not pair might get lost during anaphase segregation and this would explain the numbers of $2n = 50-59$ observed at anaphase in some of the natural, hexaploid hybrids.

This theory, however, assumes that non-reduction has only occurred in *C. littorale* since no hexaploid hybrids resembling *C. erythraea* have been found, although it is possible that they might be formed but do not survive in the wild.

It seems more likely that non-reduction occurred in a hybrid plant, since natural and artificial, tetraploid F_1 hybrids are almost sterile owing to meiotic irregularities. Furthermore, it has been shown that neither selfing the artificial F_1 hybrids nor crossing between them increases their fertility so there would be a strong selective advantage for genotypes that could overcome their sterility by some other means.

It has been shown that artificially backcrossing the natural, almost sterile, tetraploid hybrids from Ainsdale to *C. erythraea* produces tetraploid plants closely resembling this species in their morphology, and with regular meiosis and high fertility. Such plants closely resemble the fertile,

tetraploid hybrids from St Annes, W. Lancs. (Hybrid-index 15–20), and so these plants may have originated in this way. By this method, the fertility of the hybrids has been increased at the tetraploid level.

However, it has also been shown that artificially backcrossing the same Ainsdale hybrids to *C. littorale* does not increase the fertility at the tetraploid level, all such backcrosses being almost sterile. If, therefore, fertilization occurred between a non-reduced, tetraploid hybrid gamete and a normal, reduced, diploid gamete of *C. littorale*, the more fertile hexaploid genotypes might be at a selective advantage. By this method, the hexaploids would resemble *C. littorale* more closely in their morphology and pairing at metaphase and anaphase segregation would also be the same as it would have been if non-reduction had occurred in *C. littorale*.

Experiments have also shown that crossing the hexaploid hybrids with either of the tetraploid parents, or with the tetraploid hybrids, produces a few almost sterile, pentaploid plants of varying morphology, none of which has been found in the wild. It seems, therefore, that the hexaploids are isolated from their tetraploid parents and all tetraploid hybrids by a difference in chromosome number, and so in order to persist must either self or cross with each other. As has already been shown, the hexaploids are characterized by a more or less regular meiosis and good seed-set; they also breed true.

Since these hexaploids are as abundant as their parents in certain populations at Ainsdale, Freshfield and Hightown, S. Lancs., and since they breed true and are also isolated from their parents by a difference in chromosome number, they should be considered to constitute a new species. Their nomenclature will be discussed in the third and final paper in this series. This study has consequently shown that, in addition to introgression, which might have been expected, hybridization between *C. erythraea* and *C. littorale* has resulted, in certain areas, in the evolution of a new species.

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