SOME STUDIES IN *CALYSTEGIA* : COMPATIBILITY AND HYBRIDISATION IN *C. SEPIUM* AND *C. SILVATICA*

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ABSTRACT

The history of the two species, Calystegia sepium (L.) R. Br. and Calystegia silvatica (Kit.) Griseb. (= C. sylvestris (Willd.) Roem. et Schult.), in this country is outlined, and the merits of the characters variously employed to separate them are assessed. A numerical method, a modified Anderson Hybrid Index, is devised to enable the two species to be easily separated and any hybrids present to be recognised. Results are presented for seventy-two colonies, twelve of which were found to be intermediate between the two species. Pollination experiments were performed on various colonies of both species and the intermediates. Results given show that all colonies are totally self-incompatible, and that interspecific as well as intraspecific crosses can be successfully performed in any combination of taxa. The intermediates are thus probable hybrids between C. sepium and C. silvatica. Literature research showed that the hybrid had been previously known under several names, and that its correct name is $C. \times lucana$ (Tenore) G. Don. Both species and the hybrid to be commonly fertile in the field, from naturally incurred open pollination. Pollen tube and seed germination studies are described.

The significance of this system with two self-incompatible freely hybridising species producing a fully fertile F_1 generation is briefly discussed.

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1. INTRODUCTION

The presence of a related alien species of *Calystegia* (*C. silvatica*) in this country, in addition to the native *C. sepium*, has been pointed out by Lousley (1948)*. The first record of *C. sepium* is that of W. Turner for 1548 in 'The Names of Herbes' (Clarke, 1897), and the earliest British specimen of *C. silvatica* is one from Middlesex, in 1867, in the Kew Herbarium (Lousley, 1948), although Brummitt & Heywood (1960) refer this to *C. pulchra*. The alien species is a native of south and south east Europe from south Spain eastwards to the Caspian Sea and of north Africa, being sympatric with *C. sepium* for much of its range. *C. silvatica* is widespread in Britain at present, and is commoner in the north than is *C. sepium* (Tutin, 1952). In most urban areas of south-east England it is by far the commoner species, but may be scarcer than *C. sepium* in rural habitats. It mostly occurs in waste places, hedges and shrubberies, where it may accompany *C. sepium*, but it does not seem especially common in marshy places where *C. sepium* is so typical, a fact also noted by Pospichal (1899).

The two species differ by fairly distinct characters which have been summarised with varying degrees of accuracy by several workers (Lousley (1948); Stearn (1951); Tutin (1952)).

Walters & Webb (1956) and Walters & Martin (1958) point out that intermediates occur in the neighbourhood of Cambridge, and they presume these to be hybrids. Although Dandy (1958) includes the hybrid in his 'List of British Vascular Plants,' Brummitt &

*C. silvatica was recorded by Praeger (1934, p. 420) from near L. Gill, Co. Sligo, Ireland.

Heywood (1960) found 'little evidence of intermediates between them there (where they are sympatric) or in this country.' Mainly on this basis they keep the two species apart, reversing the opinion of Tutin (1959) who treated *C. silvatica* as a subspecies of *C. sepium*.

Walters & Webb (1956), Baker (1957) and Walters & Martin (1958) seem agreed that both C. sepium and C. silvatica are self-incompatible, and that larger populations set seed a great deal more readily than do small populations, the former possessing some degree of genetic heterogeneity whilst the latter are usually single clones.

Work was undertaken during the summers of 1958, 1959 and 1960 on the problems outlined above, by far the most of it being conducted at Tunbridge Wells, Kent (v.c.16). Although by no means all pink-flowered plants found were considered referable to *C. pulchra* Brummit & Heywood (= *C. dahurica* sensu Walters), all were entirely omitted from this study, since opinions are by no means in agreement as to the correct status of these forms.

2. Identification of Hybrids

In order to distinguish between the two species, *C. sepium* and *C. silvatica*, and to identify any hybrids which might be found, a numerical method was devised : a modified Anderson Hybrid Index (Anderson, 1949).

The characters by which the two species differ may be summarised under the following headings :—

1. Size. C. silvatica is larger in almost all of its parts than is C. sepium. Stearn (1951) gives a list of the average lengths of various parts of the two species, but his figures do not indicate the ranges encountered. A list of these size differences will be found in Table 4.

2. Bracteole Shape. The bracteoles in C. sepium are ovate-lanceolate to ovate, acute to obtuse and rather flat. The midrib of each bracteole is not very conspicuous, being in the same plane as the rest of the bracteole. In some instances the midrib is rendered more conspicuous by becoming raised from the plane of the rest of the bracteole to give a keeled structure, especially noticeable at the base. The margins of the bracteoles are not or are very slightly wrapped around the sides of the flower, so that in side view the calyx is clearly visible usually right to the base. The margins of the bracteoles may be undulate (Fig. 1), In C. silvatica the bracteoles, when opened out flat, are very broadly ovate, and are obtuse or sometimes mucronate. The bracteoles are not flat but greatly inflated, and are wrapped right around the sides of the flower, rendering the calyx almost or completely invisible. In addition the midrib at the base of each bracteole is conspicuously raised so that the bracteoles are pouched or saccate. An element of asymmetry not found in C. sepium is present in C. silvatica since, where the edges of the two bracteoles meet and overlap, the same bracteole overlaps the other on each side. Thus one bracteole is larger than the other (Fig. 2).

3. Relative lengths of Stamens and Styles. The five anthers are closely adjacent to the style in *Calystegia*. In *C. silvatica* the base of the stigma lobes is always well clear of the tip of the anthers, but in *C. sepium* there is usually some and often considerable overlap, although the stigma is always clear of the stamens to some degree. Stearn (1951), however, states the opposite, since he says that the style and stamens of *C. sepium* are of the same length, but in *C. silvatica* the stamens are longer than the style.

4. Leaf Shape. Various differences are given in diverse accounts, and Scholz (1960) gives distinguishing drawings. Pospichal (1899) and Hegi (1927), however, tend to indicate that the entire range of shape is found within C. sepium, which is divided into two varieties partly on this feature. Although average leaf-shapes may be at variance between the two species, there is a good deal of overlap.

5. Corolla Shape. Although C. silvatica always has the margins of the corolla turned outwards so that there is a wheel-like brim (and the corolla is vaguely trumpet-shaped), C. sepium may also show this condition to varying extents. Other plants of C. sepium lack this character, having a straight-sided cone-shaped corolla. Pospichal (1899) and Hegi (1927) use this as a further feature for distinguishing the two varieties of C. sepium.



Figs. 1–3. Bracteoles of *Calystegia. a & b.* Two bracteoles from different views. c. Diagrammatic crosssection of bracteoles. d. Larger bracteole flattened out. Fig. 1. (top) C. sepium Fig. 2. (middle) C. silvatica. Fig. 3. (bottom) C. sepium \times C. silvatica.

6. Capsule Shape. According to Tutin (1952) the smaller capsule of C. sepium is subglobose, whilst the capsule of C. silvatica is ovoid and acute.

7. Seeds. According to Tutin (1952) the larger seeds of C. silvatica are triangular-ovoid and not wrinkled, whilst those of C. sepium are more or less round but wrinkled. Further reference is made to fruit characters in Section 4.

Although the differences between the two species appear to be well marked, considerable confusion between them has occurred in the past. This is illustrated by the fact that *C. silvatica* was an escape in this country for at least eighty years before detection, and even in floras which recognised both species errors crept in. The diagram 3052a of Hegi (1927) appears not to be of *C. sepium* as it claims, but is according to Scholz (1960) of *C. silvatica*. It appears to the author to be closer to *C. silvatica* than to *C. sepium*, but closer still to the hybrid. In any case, the drawing is not a good one. Contrasting with this, Hutchinson's excellent drawing (1945) leaves one in no doubt that *C. silvatica* is depicted, although it is referred to *C. sepium*. This work appeared before *C. silvatica* was noticed in this country, however. Because of this confusion, considerable effort was expended in selecting the best characters for species separation.

The value of the size of various parts varies greatly from character to character, but the length of corolla and width of bracteoles were found to be the best or easiest characters to work with. Capsule shape was not used, as this would have necessitated two visits to each colony to be studied, which in many cases was not possible. In addition, capsules with less than the maximum of four seeds have a less globose shape, in extreme cases the one-seeded capsule of either species being ovoid-lanceolate. Seeds, it is thought, should be used on no account in *Calystegia*, since it is self-incompatible (see below). Seeds must be the product of two distinct clones and, although testa markings could be thought of as female alone, the shape of the seed may well be affected by the hybrid embryo inside. Since leaf and corolla shape are both too variable, and the style/stamen ratios differ but very slightly between the two species, bracteole shape was used as the only other criterion. This was measured in two ways, partly since the two methods each measured different and to some extent independent variables, and partly because size, in which two variables were measured, is considered no more important than is bracteole shape.

The precise nature of the four variables measured is as follows :---

1. Corolla length (c). Length from extreme tip of pedicel to tip of corolla.

2. Bracteole width (w). Maximum width of the bracteoles (the larger of the two if different) when flattened out. In C. silvatica and some C. sepium the midrib has to be cut longitudinally at the base to enable the bracteole to be flattened out (Figs. 2–3).

3. Bracteole inflation (w/e). The ratio of bracteole width (above) to e. This latter measurement is the width of the bracteoles in their natural state, not flattened out. Thus the lowest possible value is unity. (Figs. 2–3).

4. Bracteole midrib conspicuousness (m/e). The ratio of m, the distance between the midribs of the two bracteoles measured some 2-3 mm. from the base, to e (above). In C. sepium the value is nearly always less than unity, and in C. silvatica always over unity (Figs. 1-3).

It is obvious from the above that each of the four independent variables have been so arranged as to be higher in *C. silvatica* than in *C. sepium*.

These four variables were measured or calculated for six flowers randomly taken from each 'colony.' Abnormal flowers which are occasionally produced were not used. These include : flowers with abnormally small corollas (produced in the already occupied axils of some leaves late in the year); flowers with extra bracteoles (either supernumerary or modified from sepals); flowers with an extra flower in the axil of one or both bracteoles; or flowers with extra bracteoles or leaves subtending axillary flowers borne on the pedicel.

Each of the four variables was scaled to a scale of 1-10 which was constructed to embrace the entire range encountered (Table 2). This bestows equal significance to each of the four characters. The scaled figure for each variable was averaged for the six flowers, and the four averaged scaled figures were totalled. This totalled averaged scaled figure (*Ts*) was used as a character index for each colony, and is seen to run in a scale from 4 to 40. Smallest specimens of *C. sepium*, having an averaged scaled figure of one for each of the four variables, will have a *Ts* of four, whilst largest specimens of *C. silvatica* will have ten for each variable and a total of forty. The extremes actually encountered were six and thirty-five.

At first, however, two very large colonies were investigated in a slightly different manner, in order to ascertain the type of variation to be encountered. One colony (colony 72) was obviously polymorphic, appearing to the eye to consist of *C. sepium* and *C. silvatica* mixed, with no intermediates. The colony covered at least 1,000 square yards, and separation into distinct plants was not possible. Thus one flower from each branch (and there is nearly always only one in flower at a time) was treated as if a separate plant. Its *Ts* value was calculated (no averaging being here involved) and the *Ts* values of the forty-four flowers measured plotted. The results are shown in Fig. 4. Reference to this figure will show at once that two distinct taxa are present, with no intermediates. These two, *C. sepium* and *C. silvatica*, have *Ts* ranges of 7–11 and 23–29 respectively. As well as examining many known colonies in this way to make certain that the hybrid index constructed always afforded a good separation of *C. sepium* and *C. silvatica*, whether either



Fig. 4. Hybrid index of individual flowers of colony 72.



Fig. 5. Hybrid index of individual flowers of colony 71.



Fig. 6. Hybrid index of individual colonies of colonies 1-70.

one or both species were present in the same colonies, I searched the literature in order to gain some idea of the range of structure to be encountered throughout the whole of Europe and North Africa in the *C. sepium – silvatica* complex. It was concluded that the extreme ranges of structure described and found in the two species were quite narrow enough to fit into the present hybrid index, and still to allow the two species to be easily separated. This indicates that the four variables used are in fact suitable for species separation.

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W	SW	w/e	sr_1	е	т	m/e	sr ₂	С	SC	Ts
45	10	2.36	7	19	23	1.21	5	72	8	30
41	10	2.41	8	17	22	1.30	5	74	8	31
38	9	2.24	7	17	21	1.24	5	72	8	29
34	8	2.12	6	16	18	1.12	4	68	7	25
39	9	2.16	6	18	22	1.22	5	73	8	28
28	6	2.00	6	14	18	1.28	5	64	6	23
53	10	2.30	7	23	27	1.18	4	80	10	31
35	8	2.33	7	15	22	1.47	6	75	9	30
32	7	2.46	8	13	20	1.54	6	71	8	29
22	4	2.20	7	10	15	1.50	6	68	7	24
30	6	2.30	7	13	17	1.31	5	70	8	26
31	7	2.07	6	15	20	1.30	5	74	8	26
18	3	1.28	2	14	8	0.57	1	50	4	10
17	3	1.31	2	13	10	0.77	2	54	4	11
16	2	1.23	2	13	8	0.62	2	48	3	9
13	2	1.30	2	10	7	0.70	2	49	3	9
15	2	1.15	1	13	9	0.69	2	49	3	8
17	3	1.31	2	13	9	0.69	2	48	3	10
14	2	1.27	2	11	7	0.64	2	45	3	9
14	2	1.27	2	11	8	0.73	2	47	3	9
15	2	1.25	2	12	7	0.58	1	46	3	8
14	2	1.27	2	11	9	0.82	3	44	2	9
18	3	1.13	1	16	6	0.38	1	48	3	8
25	5	2.27	7	11	15	1.36	5	54	4	21
22	4	1.84	5	12	13	1.08	4	51	4	17
18	3	2.00	6	9	10	1.11	4	33	1	14
20	4	1.54	3	13	13	1.00	4	56	5	16
20	4	1.82	5	11	14	1.27	5	53	4	18
24	5	2.00	6	12	14	1.17	4	56	5	20
29	6	2.42	8	12	16	1.33	5	70	8	27
24	5	2.40	8	10	15	1.50	6	64	6	25
26	5	2.36	7	11	16	1.48	6	69	7	25

 TABLE 1.

 Data compiled from Colony 71.

Key to Letters (Lengths in mm.)

w = Bracteole Width (Flattened out).	sr_2 = Scaled Value of m/e .
sw = Scaled Value of w .	c = Corolla Length.
e = Bracteole Width (Natural).	sc = Scaled Value of c .
m = Distance apart of Midribs.	$Ts = sw + sr_1 + sr_2 + sc.$
$sr_1 = $ Scaled Value of w/e .	

The second very large colony, colony 71, differed from the last in that, to the eye, intermediates were apparently present. Otherwise the two colonies were rather similar and were treated alike. Table 1 represents the measurements and calculations for colony 71, and Fig. 5 the plotted Ts values. It will be seen that, as casual inspections strongly suggested, intermediates between the two species do occur in colony 71. The ranges of *C. sepium*, the intermediates and *C. silvatica* are 8–11, 14–21 and 23–31 respectively.

Colonies 1–70 were relatively isomorphic in nature and wherever pollination experiments were undertaken (see below) they proved to be uniclonal. They were always spatially isolated from other colonies, to varying degrees. Table 3 represents the measurements and calculations of just six of the seventy colonies, which are seen to be treated in the manner

TABLE 2.

Scaled equivalents of the four variables.

1 – Corolla Length	(c)	2-Bracteole Width (w)				
Scaled Number	Measurement	Scaled Number	Measurement			
1	-39 mm.	1	-12 mm.			
2	40–44 mm.	2	13–16 mm.			
3	45–49 mm.	3	17–19 mm.			
4	50–54 mm.	4	20–23 mm.			
5	55–59 mm.	5	24–26 mm.			
6	60–64 mm.	6	27–30 mm.			
7	65–69 mm.	7	31–33 mm.			
8	70–74 mm.	8	34–37 mm.			
9	75–79 mm.	9	38–40 mm.			
10	80– mm.	10	41– mm.			
3 – Bracteole Inj	flation (w/e)	4 – Bracteole Midrib	Conspicuousness (m/e)			
3 – Bracteole Inj Scaled Number	flation (w/e) Ratio	4 – Bracteole Midrib Scaled Number	Conspicuousness (m/e) Ratio			
3 – Bracteole Inj Scaled Number 1	Aation (w/e) Ratio 1·00–1·19	4 – Bracteole Midrib Scaled Number 1	Conspicuousness (m/e) Ratio –0.59			
3 – Bracteole In Scaled Number 1 2	flation (w/e) Ratio 1·00–1·19 1·20–1·39	4 – Bracteole Midrib Scaled Number 1 2	Conspicuousness (m/e) Ratio -0.59 0.60-0.79			
3 – Bracteole In Scaled Number 1 2 3	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59	4 – Bracteole Midrib Scaled Number 1 2 3	Conspicuousness (m/e) Ratio _0.59 0.60-0.79 0.80-0.99			
3 – Bracteole In Scaled Number 1 2 3 4	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59 1·60–1·79	4 – Bracteole Midrib Scaled Number 1 2 3 4	Conspicuousness (m/e) Ratio -0.59 0.60-0.79 0.80-0.99 1.00-1.19			
3 – Bracteole In Scaled Number 1 2 3 4 5	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59 1·60–1·79 1·80–1·99	4 – Bracteole Midrib Scaled Number 1 2 3 4 5	Conspicuousness (m/e) Ratio -0·59 0·60-0·79 0·80-0·99 1·00-1·19 1·20-1·39			
3 – Bracteole In Scaled Number 1 2 3 4 5 6	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59 1·60–1·79 1·80–1·99 2·00–2·19	4 – Bracteole Midrib Scaled Number 1 2 3 4 5 6	Conspicuousness (m/e) Ratio -0·59 0·60-0·79 0·80-0·99 1·00-1·19 1·20-1·39 1·40-1·59			
3 – Bracteole In Scaled Number 1 2 3 4 5 6 7	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59 1·60–1·79 1·80–1·99 2·00–2·19 2·20–2·39	4 – Bracteole Midrib Scaled Number 1 2 3 4 5 6 7	Conspicuousness (m/e) Ratio -0·59 0·60-0·79 0·80-0·99 1·00-1·19 1·20-1·39 1·40-1·59 1·60-1·79			
3 – Bracteole In, Scaled Number 1 2 3 4 5 6 7 8	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59 1·60–1·79 1·80–1·99 2·00–2·19 2·20–2·39 2·40–2·59	4 – Bracteole Midrib Scaled Number 1 2 3 4 5 6 7 8	Conspicuousness (m/e) Ratio -0.59 0.60-0.79 0.80-0.99 1.00-1.19 1.20-1.39 1.40-1.59 1.60-1.79 1.80-1.99			
3 – Bracteole In, Scaled Number 1 2 3 4 5 6 7 8 9	flation (w/e) Ratio 1·00–1·19 1·20–1·39 1·40–1·59 1·60–1·79 1·80–1·99 2·00–2·19 2·20–2·39 2·40–2·59 2·60–2·79	4 – Bracteole Midrib Scaled Number 1 2 3 4 5 6 7 8 9	Conspicuousness (m/e) Ratio -0.59 0.60-0.79 0.80-0.99 1.00-1.19 1.20-1.39 1.40-1.59 1.60-1.79 1.80-1.99 2.00-2.19			

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Colony 3	(C. sepium ×	C. silve	atica)							
W	sw	w/e	Sr ₁	е	т	m/e	Sr.	с	SC	Ts
16	2	1.60	4	10	14	1.40	6	55	5	
15	2	1.50	3	10	13	1.30	5	50	4	
14	2	1.40	3	10	14	1.40	6	47	3	
17	3	1.70	4	10	15	1.50	6	48	3	
14	2	1.40	3	10	13	1.30	5	51	4	
15	2	1.67	4	9	13	1.44	6	49	3	
Mean	2		3.5				5.7		3.7	15
Colony 8	(C. sepium)									
W	SW	w/e	sr_1	е	т	m/e	sr_2	С	SC	Ts
15	2	1.25	3	12	6	0.20	1	38	1	
14	2	1.40	3	10	5	0.20	1	38	1	
14	2	1.27	2	11	8	0.73	2	40	2	
16	2	1.23	2	13	9	0.69	2	41	2	
14	2	1.16	1	12	10	0.83	3	44	2	
15	2	1.12	1	13	8	0.61	2	45	3	
Mean	2		2				2		2	8
Colony 9	(C. silvatica)									
W	sw	w/e	sr ₁	e	m	m/e	sr ₂	c	SC	Ts
28	6	2.15	6	13	20	1.54	6	68	7	
30	6	2.30	1	13	18	1.38	2	61	6	
23	4	2.10	0	11	10	1.46	6	64	6	
20	6	2.30	6	13	20	1.42	5	62	0	
20	6	2.00	6	14	10	1.36	5	68	7	
Mean	5.7	215	6.3	14	19	1 50	5.5	00	6.5	24
Colony 11	(C siluation	`			÷		~			
w	(C. silvatica) wle	Sr.	P	m	mle	Sr.	C	sc	T_{S}
32	7	2.46	8	13	17	1.31	5	65	7	15
31	7	2.58	8	12	17	1.42	6	63	6	
27	6	2.45	8	11	15	1.36	5	64	6	
31	7	2.38	7	13	19	1.46	6	64	6	
31	7	2.58	8	12	17	1.42	6	60	6	
28	6	2.80	10	10	17	1.70	7	67	7	
Mean	6.7		8				6		6.3	27
Colony 30	(C. sepium)									
w	SW	w/e	sr ₁	е	m	m/e	sr_2	С	SC	Ts
12	1	1.20	2	10	7	0.70	2	50	4	
13	2	1.30	2	10	7	0.70	2	50	4	
11	1	1.38	2	8	8	1.00	4	50	4	
15	2	1.50	3	10	8	0.80	3	53	4	
13	2	1.45	3	9	7	0 ·78	2	53	4	
11	1	1.38	2	8	5	0.62	2	53	4	10
Mean	1.2		2.3				2.5		4	10
Colony 66	(C. sepium	\times C. sil	vatica)							
W	SW	w/e	sr ₁	е	m	m/e	Sr ₂	С	SC	Ts
22	4	1.57	3	14	14	1.00	4	54	4	
22	4	1.69	4	13	14	1.08	4	54	4	
24	5	1.85	2	13	14	1.10	4	50	5	
19	3	1.03	4	11	13	1.10	4	30	2	
25	4	2.08	5	12	13	1.00	4	49	э 1	
Mean	4	2 00	1.5	14	13	1 00	4	55	+	17
1710GII	7		тJ				- T		т	17

TABLE 3. Data compiled from 6 of the 70 small colonies.

Watsonia 5 (2), 1961.

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first described. The Ts values are plotted in Fig. 6: here each individual value represents a colony rather than a flower. As might well be expected, the graph in Fig. 6 shows considerable similarity to that in Fig. 5. Two main peaks, representing the two species, are present, together with numerous intermediates of varying character.

It seems likely that the intermediates shown in Figs. 5 and 6 are hybrids, C. sepium \times C. silvatica, although stronger evidence for this assumption will be brought forward below.

3. POLLINATION AND GERMINATION STUDIES

Having obtained an accurate method of separating the two species and the putative hybrid, I carried out a series of pollination experiments on these three taxa. Advantage was taken of the regular flower opening sequence in *Calystegia*, a flower being in bud one day, in flower the next, and withered the next. Large buds, ready to open the following day, were enclosed in pollen-proof bags made of grease-proof paper (polythene and cellophane being rejected to alleviate condensation). Later a quicker method was employed, whereby the tip of the corolla was tied with knitting wool to prevent it from opening, the corolla itself acting as the pollen-proof bag. With intended cross-pollinations, as opposed to selfpollinations, emasculation was usually effected just prior to enclosure in the pollen-proof bag. This entailed baring the inner parts of the flower and excising the anthers, the filaments being left intact to prevent unnecessary damage. Emasculation was not always carried out, however, as *Calystegia* was known to be self-incompatible, both from the literature and from previous personal experience. However, where it was not effected, self-pollination was prevented wherever possible by supporting the flower in its bag with the apex uppermost (the stigma being distal to the anthers). Anther dehiscence occurs almost as soon as corolla opening, and is neither introrse nor extrorse, but lateral (although Warnstorf (Knuth, 1909) says it is extrorse). When the flowers had opened on the second day pollination was effected. Pollen from the chosen source was smothered on to the inside and outside of the two stigma lobes with the aid of a pencil point, after which the bag or wool was replaced. Each colony used as a pollen source was designated a colour, and a ribbon of that colour was tied on to the pedicel of all flowers pollinated from that source. Wherever possible reciprocal pollinations were effected, and pollen from as many sources as possible was used for the various flowers of each colony used as a female parent.

With three taxa, there are twelve possibilities of pollination : three selfings, three intraspecific crosses and six interspecific crosses. In addition self-pollinations were effected by pollination from one flower on to another known certainly to be on the same plant. All fifteen of these possibilities were carried out, and the results are briefly summarised below.

		the second s	And in case of the local division of the loc
Selfi	ngs	Attempts	Successes
1.	sepium flowers selfed	26	0
2.	sepium flowers of the same plant crossed	5	0
3.	Hybrid flowers selfed	9	0
4.	Hybrid flowers of the same plant crossed	10	1
5.	silvatica flowers selfed	12	0
6.	silvatica flowers of the same plant crossed	8	0
Intro	aspecific Crosses		
7.	sepium onto sepium	28	20
8.	Hybrid onto hybrid	12	8
9.	silvatica onto silvatica	14	13
Inte	rspecific Crosses (Hybridisations)		
10.	Hybrid onto sepium	10	6
11.	silvatica onto sepium	12	8
12.	sepium onto hybrid	8	4
13.	silvatica onto hybrid	8	3
14.	sepium onto silvatica	21	11
15.	Hybrid onto silvatica	8	2

As may be seen from the above results, all three taxa are self-incompatible. Although one of the seventy self-pollinations surprisingly proved fertile, it is possible that this was the result of contamination by compatible pollen, especially as it involved transference of pollen from one flower to another rather than from the anthers to stigma of the same flower. On the other hand it could indicate that *Calystegia* is incompletely self-incompatible. All intraspecific and interspecific pollinations, in all nine of the possible combinations, proved successful to some extent, though very variously so. Although figures for interspecific pollinations are mostly the lower of the two sets, little significance should be attached to this as in many cases different colonies were involved, and the figures represent the sums of three seasons' work. The figures show that the two species are highly interfertile and that the hybrid is fertile in crosses with other hybrids and both parents, on both male and female sides. These results are considered extremely strong evidence that the intermediates between the two species, *C. sepium* and *C. silvatica*, are in fact hybrids.

The biological nature of some populations was later investigated, the results fully confirming those of Walters & Martin (1958). Small colonies, isomorphic to the eye, proved to be single clones since all combinations of pollinations proved unsuccessful. Larger colonies, nearly always polymorphic to the eye to some degree, were composed of two or more clones, since cross-pollination in certain directions produced good fertile seed. Some fairly large (isomorphic) colonies, however, proved to be single clones. Seed-set in *Calystegia* has been observed to be good in the area of study during the years 1958-60. Populations of either species and of all types of intermediates commonly bear very good quantities of seed. As Baker (1957) points out, small populations of a single clone are less likely to produce seed than are large multiclonal populations, and Baker observed that in the London area the small colonies were frequently sterile or sparsely fertile, whilst larger colonies not far distant had good seed-set. For some reason this situation appears not to exist in the present area of study, since almost all colonies produced good seed-set. In some of the larger colonies, which superficially appeared to have a better seed-set than the smaller colonies, exact counts showed that the total number of seeds produced, expressed as a percentage of the total number of seeds possible, was no larger than on many of the small uniclonal plants, which in extreme cases merely consisted of a single branch with no more than ten flowers. There are ideally and at the most four seeds per capsule in Calvstegia. Hegi (1927) states ' seeds 4, rarely 3,' but this certainly appears to be an optimistic report. According to the number of ovules fertilized there may be any number of seeds from nil to four, capsules with one or two seeds being guite frequent, but those with nil, three or four more so. The number of seeds per capsule did not vary from species to species, nor from hybrid to species, and as far as could be ascertained the hybrid and its back-crosses appeared to be equally as fertile as the two parental species. I have found one capsule of C. sepium with five good seeds.

The unsuccessful self-pollinations soon made themselves quite evident as the whole flower structure, bracteoles and distal end of the pedicel had turned brown and had shrivelled by early in September. Observation on the pollen tubes of pollen on the stigma of the same flower was therefore made. Flowers were self-pollinated in the usual way, and the style and stigma removed twelve, twenty-four, thirty-six and forty-eight hours after pollination. The styles were boiled in cotton blue in lactophenol for about three minutes, when the dye stained all parts and the lactophenol entered and softened the tissues. The styles, then a tangled, limp mass, were removed and washed in lactophenol and each was squashed flat on a slide under a coverslip and ' tapped out.' Under the microscope the pollen grains and tubes appeared a dark intense blue, and the stylar and stigmatic tissue a pale blue. In all cases the pollen tubes, if present, were short and contorted, and had never penetrated more than a fractional distance into the stigma. In cases where compatible pollen had been deposited on the stigma, pollen tubes were seen to be long and straight, entering the stylar tissue. Ouite obviously, assuming incompatibility to be of the normal type (Lewis, 1954), the inhibitory reaction, or lack of stimulatory reaction, (as the case may be) occurs in the stigma. Excision of the stigma did not allow self-pollination to result in fertilization, however, but the results of this experiment must be regarded as inconclusive as

yet. It is now generally considered that stigmatic inhibition indicates a sporophytic incompatibility system.

Peters (Hegi, 1927) found that the pollen of *C. sepium* is very sensitive to damp, and that in foggy conditions 50% of the grains burst. He could not get the grains to germinate away from the stigmata, glucose, sucrose and even stigma extracts not providing the essential medium. These observations were confirmed with both species on several occasions : strong glucose solutions caused plasmolysis; very weak solutions caused bursting (as did water); and intermediate solutions had no effect at all. On all plants examined the percentage of grains which appeared to be fertile (full of contents, spherical and staining black with iodine) was over 95%.

Using seeds collected from natural pollinations, attempts were made to find the best time to germinate the seeds. Three types of seed were used : white soft seeds not yet really ripe, although in brown capsules; hard, dark brown, fully ripe seeds; and similar seeds chipped with a scalpel. After about three days all the white soft seeds had germinated; the hard unchipped seeds had not yet imbibed water; and the chipped seeds provided mixed results, some germinating freely, others not, but in all cases the hard testa in some way impeding the unfolding of the cotyledons. In *Calystegia* germination is epigeal, two thin green ovate cotyledons being raised on a fairly long hypocotyl. In all seeds examined an embryo was present, and if there was no germination it was prevented either by the hard testa or by attacks from mould. Exactly similar results were obtained with seeds produced by experimental intraspecific and interspecific pollinations. Thus the presence of ripe seed may be taken to indicate a successful pollination. In artificial pollinations rather more fruits had one or two seeds than was the case in natural pollinations.

In natural conditions the seeds probably fall to the ground, taking all winter for their testa to be fully water-saturated and thus made pliable. Special adaptations for seed dispersal appear to be more or less absent, since the capsules have no explosive device, and the four sutures are not lines of dehiscence. Irregular pole-to-pole cracks appear in the capsule and many seeds drop out by reason of shaking caused by air currents. However, many seeds remain trapped in their old capsules for at least a year, and ultimately they probably rot away with the capsule wall. However, in 1946 Toole & Brown reported that after 39 years over half of the seeds of *C. sepium* were still viable (Salisbury, 1961).

4. DISTRIBUTION AND DESCRIPTION OF THE HYBRID

From the previous sections it seems highly probable that hybrids between *Calystegia* sepium and C. silvatica occur in this country, and these hybrids are fertile, forming F_{2} hybrids as well as backcrosses with either parent. Twelve such colonies were found in the neighbourhood of Tunbridge Wells, Kent, v.c. 16. One British record only has apparently been published - namely ' in the vicinity of Cambridge,' v.c. 29 (Walters & Martin, 1958), but it seems probable that the hybrid does or will occur wherever the two species are in close proximity. Dr. W. T. Stearn informs me that intermediates occur with both parents by the Thames at Kew (Surrey, v.c. 17). In the Kew herbarium specimens are present from : Alderney, v.c. S (Jackson, 1932); Surrey, v.c. 17 (Clarke, 1901; Summerhayes & Milne-Redhead, 1932; Turrill, 1956); Berkshire, v.c. 22 (Elliot, 1945); Huntingdonshire, v.c. 31 (Sandwith & Gilbert, 1958); E. Gloucestershire, v.c. 33 (Riddelsdell, 1934); Glamorganshire, v.c. 41 (Wade, 1947); and Merionethshire, v.c. 48 (Milne-Redhead, 1948). Thus 1901 appears to be the first British record. The earliest material of the hybrid seen by the author (and which also pre-dates any published record) is a specimen labelled 'Anglia, Herb. Forsyth' in the handwriting of Hooker (senior) at the Kew herbarium. It is on the same sheet as a specimen of C. sepium collected in France by Hooker in 1819, and it is also labelled C. sepium. The hybrid is most probably of the same period as the other specimen, and of garden origin.

In order to obtain an idea of the foreign distribution of the hybrid, the ranges of the two parents are clearly of some significance. *C. sepium*, in one form or another, appears

to be pan-North-Temperate, so hybrid distribution will be limited only by that of C. silvatica. C. silvatica is a native of south Europe from S. Spain and Portugal (?) eastwards to the Caspian Sea. Although neither Lousley (1948) nor Tutin (1952) mention north Africa in its area of distribution, it is obviously native there. It is mentioned in all the thorough floras of north-west Africa (Battandier, 1890; Sauvage & Vindt, 1954; Murbeck, 1905; and others), and specimens from Morocco and Algeria are present in the British Museum dating from the first half of the nineteenth century. Its range in this continent appears to be throughout most of Morocco (including Tangier), Algeria and Tunisia. It is also found in Malta (Borg, 1927), but it has not been recorded from the Balearics. These older literature records, however, should probably not be used without further confirmation. C. silvatica is now naturalised in various more northerly areas of Europe (Scholz, 1960), as it is in Britain. A specimen in the British Museum herbarium from Portugal (1954) may be a native or naturalised example.

In investigating the nomenclature of the *C. sepium-silvatica* complex a variety of names is encountered. Although several authors have attempted to split off species from *C. silvatica* as here understood, this does not seem to be possible. The two usual synonyms are *C. sylvestris* and *C. inflata*. Pomel (1876) described a species which he named *C. physoides* from North Africa. Pau (1924) described a variety of *C. sepium* (var. *tangerina*) which is clearly a synonym of *C. physoides*, and was treated as such by subsequent authors (e.g. Sauvage & Vindt, 1954). Moreover, examination of Pomel's description of *C. physoides* brings one to the conclusion that this taxon cannot be separated from *C. silvatica*, at least specifically. This conclusion was also reached by Battandier (1890), who gave *C. physoides* as a synonym of *C. silvatica* Griseb. The present author, then, considers that there is but one species in the *C. silvatica* group, which, as Dandy (1958) concluded, is to be referred to as *C. silvatica* (Kit.) Griseb. Even more names are to be associated with the *C. sepium* group, although most of these have been treated as varieties rather than as separate species. Pomel (1876), however, described *C. obtusa* from N.W. Africa, which should probably be included with *C. sepium*.

If all the names involved in the C. sepium-silvatica complex which are referable to either C. sepium (L.) R. Br. or to C. silvatica (Kit.) Griseb. are discarded, three names (involving two taxa) remain. The earliest of these is Convolvulus lucanus, named by Tenore (1826) from South Italy. Don (1837) transferred it to its present genus as Calystegia lucana, but Fiori (1926) reduced it to a variety of *Convolvulus sepium*, as he did *C. silvatica*. Choisy (1845) called it *Calystegia sepium* var. *tubata*, but it is clear that he refers to the same plant, as he gives the synonym, and the only specimen he cites is one sent to him by Tenore himself. Examination of the descriptions of Convolvulus lucanus given by Tenore (1826; 1824-29; and 1831, Syll.), and of the excellent plate given in his Flora Napolitana, shows clearly that his plant is *Calvstegia sepium* \times *silvatica*. The plate depicts a plant exactly intermediate between the two parents, and this fact is actually mentioned in his third cited work. The third name is Calystegia barbara, described by Pomel (1874) from North Africa, and reduced to C. sepium var. barbara by Jahandiez & Maire (1934). Pomel's description is fairly lengthy, and it appears that the plant is also a hybrid between C. sepium and C, silvatica, although perhaps closer to the former. Battandier (1890) reached the same conclusion, as he merely states under C. barbara 'intermediate between the two preceding.' The hybrid should thus be referred to as *Calystegia* \times *lucana* (Tenore) G. Don.

Thus, although Brummitt & Heywood (1960) say that they find little evidence of hybrids where C. *silvatica* is native (or elsewhere), it is not surprising to find that hybrids are localised both in the literature and as herbarium specimens, under a great variety of names. These localities are perhaps best mentioned geographically rather than chronologically :-

NORTH AFRICA – the first record for this continent is that of Pomel (1874), who gives several localities in Algeria, which are repeated, with one addition, by Battandier (1890). A good specimen from Algeria (Gandoger, 1879) is also in the British Museum herbarium, labelled as *C. sagittata*. Sauvage & Vindt (1954) record the hybrid from Morocco, giving one locality only (Beni Mellal, Jahandiez, 1925), which was previously

recorded by Jahandiez & Maire (1934) but without a date. A specimen with these exact details of collector, date and locality is in the British Museum herbarium, named *C. sepium* var. *barbara*. The specimen may be a hybrid between *C. sepium* and *C. silvatica*, but as previously implied it is much closer to *C. sepium* than to the other parent. Nomenclaturally however, it is still a synonym of *C.* × *lucana*. Ball (1878) also recorded the hybrid from Morocco, stating 'specimina nostra intermedia sunt.' A further specimen of *C.* × *lucana* from Morocco (Trethewy, 1933) is in the Kew herbarium.

RUSSIA – A single specimen from Lenkoran on the Caspian Sea in S.W. Russia is in the Kew herbarium (Hohenacker, 1838). This is probably the specimen Lousley (1948) cited as C. sylvestris when he gave the distribution as '... east to the Caspian Sea at Lenkoran.'

GREECE – A specimen from Greek Macedonia is in the Kew herbarium (Russell, 1918).

ALBANIA-A specimen from Albania is in the British Museum herbarium (Baldacci, 1898).

ITALY – Tenore (1826) described the hybrid from Italy, the type localities being Auletta and Lauria in the region Lucania in south Italy. A number of other localities are given in his five cited works, all at about the same latitude as Rome or further south. A specimen is in the Kew herbarium which was sent to J. Gay by Tenore in 1830. It is labelled by Tenore, 'Macchia Matthei e Auletta.' Which of the two localities it was collected from is not clear. Auletta (one of the type localities) is in south Italy, in Lucania, whilst Macchia Matthei is close to Rome. It is possibly some (hitherto unrecognised) sort of type specimen of Convolvulus lucanus. An additional possibility is that it is the holotype of Choisy's C. sepium var. tubata, since Choisy (1845) said that the only specimen he had seen was one sent from Italy by Tenore. There is a second specimen at Kew sent by Tenore to Gay, which Tenore also labelled Convolvulus lucanus. This specimen is clearly C. silvatica, however, so it is possible that Tenore did not have a very clear idea of his ' Convolvulus lucanus'. This specimen was sent to Gay in 1827 and is labelled 'Nella Basilicata Nel Gargano etc.' by Tenore. It is most unlikely that this was the specimen referred to by Choisy (1845), as he had a good idea of C. silvatica. Ball (1878) recorded C. \times lucana from Italy as he states ' formae intermediae praesertim in Italia occurrunt.'

SPAIN – A specimen from El Cobre, Gibraltar (Wolley-Dod, 1912) labelled *C. sepium* is in the British Museum herbarium. This is the same locality as is given in Wolley-Dod (1914) under '*C. sepium* var. *sylvestris* (?)'.

Some of the descriptions of the hybrid taxon are fairly lengthy, and Tenore's plate is most satisfactory, but measurements have never been included. A description of the hybrid is thus given here, from British material the author has seen in the field (see also Fig. 3).

CALYSTEGIA × LUCANA (Tenore) G. Don (pro sp.) (= C. sepium (L.) R. Br. × C. silvatica (Kit.) Griseb.); Convolvulus lucanus Tenore, Fl. Neap. Prod. App., 5, 9, (1826); Convolvulus sepium var. lucanus Fiori, Nuov. Fl. Anal. It., 2, 296 (1926); Calystegia lucana G. Don, Gen. Syst., 4, 296, (1837); Calystegia sepium var. tubata Choisy in DC., Prod. Syst. Nat. 9, 433, (1845); Calystegia barbara Pomel, Nouv. Mat. Fl. Atlant., 1, 83, (1874); Calystegia sepium var. barbara Jahandiez & Maire, Cat. Pl. Maroc, 3, 591 (1934).

A fertile hybrid between C. sepium and C. silvatica, capable of crossing with other similar plants and with either parent. It is intermediate between its two parents in all characters, and may be distinguished from them by : pedicels 30-100 mm. long; corolla white (always?), 41-62 mm. long; stamens 20-21 mm. long; style and stigma 20-23 mm. long; bracteoles broadly ovate, 14-25 mm. wide when flattened out, acute, obtuse or mucronate at apex, weakly cordate at base, slightly to strongly inflated, midrib very prominent especially at base, edges overlapping at each side and partially obscuring the calyx. Ratio of midrib-to-midrib (m) to edge-to-edge (e) measurement of bracteoles in natural condition 0.85-1.45.

Known from Britain, Morocco, Algeria, S.W. Russia, Greece, Albania, Italy and S. Spain.

Since not all the previous literature giving details of measurements of *C. sepium* and *C. silvatica* flowers is satisfactory, this opportunity is taken of compiling a list of the most useful taxonomic measurements of the three taxa (Table 4). The figures given in this table represent the normal ranges found in the 72 colonies studied, only very abnormal measurements being omitted, and they have been found to hold good for all the British herbarium material seen. Great caution should be exercised in applying these figures to foreign material, however.

With regard to some characters, the literature proves somewhat controversial, due probably in part to vastly different areas and times of observation. For example, Stearn (1951) gives the stamen length of *C. sepium* as 15 mm., and of *C. silvatica* 25 mm. In addition to the information given in Table 4, pollen grain sizes were also investigated. Hegi (1927) gives the diameter of *C. sepium* pollen as 76–84 μ , and Erdtman (1952) as 75–80 μ . Diameters of grains were first measured in colony 71, when it was seen that they afforded good separation of the three taxa : the diameters encountered were 67–77 μ , 77–86 μ and 82–91 μ for *C. sepium*, *C.* × *lucana* and *C. silvatica* respectively. However, it was later found that this distinction is not constant, for one colony of *C. sepium* possessed grains of 83–88 μ .

Literature regarding seed and capsule shape and size is especially diverse. The accounts of Pospichal (1899) and Hegi (1927) are very similar, the latter probably adapted from the former. Neither account refers to any substantial difference in either capsule or seed shape, and the only measurement given is ' about 5 mm.' for the seed length in *C. sepium*

	C. sepium	C. imes lucana	C. silvatica
Length of corolla	36–55 mm.	41–62 mm.	58–82 mm.
Length of pedicel	24-80 mm.	30-100 mm.	70-140 (-200) mm.
Length of stamens	17–18 mm.	20-21 mm.	23–26 mm.
Length of style + stigma	16–19 mm.	20-23 mm.	24–28 mm.
Width of bracteoles	10–18 mm.	14-25 mm.	21–45 mm.
Bracteole ratio, w/e	1.00-1.50 (-1.75)	1.40-2.40	(1.75-) 2.00-3.25
Bracteole ratio, m/e	0.40-1.10	0.85-1.45	1.15-2.20
Length of capsule	9–10 mm.		8·5–10 mm.
_	(mean $= 9.15$ mm.)		(mean = 9.60 mm.)
Width of capsule	8·5–10 mm.		9–11 mm.
	(mean = 9.40 mm.)		(mean = 9.85 mm.)
Length of capsule beak	1.5–2.0 mm.		2.5-4.0 mm.
Length of seeds	4·5−5·0 mm.		4·5–6·0 mm.
			1

 TABLE 4.

 Characteristic measurements of the 3 taxa.

(Hegi). Although a number of authors give minor differences between the fruits of the two species, none give the striking differences described by Tutin (1952). With regard to the capsule Tutin states that in *C. sepium* it is subglobose and 7–8 mm. long, whilst in *C. silvatica* it is ovoid, acute, and about 12 mm. long. Furthermore Tutin states that in *C. sepium* the seeds are 4–5 mm. long and more or less round but wrinkled, whilst in *C. silvatica* they are 6–7 mm. long, triangular-ovoid and not wrinkled. In neither case do the data coincide with those collected by the present author (Table 4). Examination of many colonies produced no constant differences in the seeds of the two species, either in shape, size or texture. With regard to the capsule, no constant size difference was found. The capsules of the two species showed a rather minor difference in most cases, the beak being larger and stouter in *C. silvatica*, and less abruptly delimited from the rest of the capsule. The overall shapes are the same in both species, however, never approaching a condition which

could be described as acute, but being broadly ovoid to subglobose, and very obtuse to truncate at the apex.

As pointed out in Section 2, it is important to use only four-seeded capsules for these considerations. Apart from the fact that a capsule with fewer than four seeds is less rounded, the seeds are quite different in shape, since the two flat faces of the seeds, which are present where the seed adjoins its two neighbours in the capsule, are absent when there are no neighbouring seeds. In extreme cases the solitary seed is quite round in section. Seeds of *C. silvatica*, however, do tend to have more conspicuously flattened faces than those of *C. sepium*, and the hilum tends to be more sunken. An additional precaution is that only absolutely ripe seeds should be used, as the apparently mature yet soft and still hydrated seeds of both species often found in brown capsules are considerably larger than in the truly mature (hard, dry) state. Although Pospichal (1899) and Hegi (1927) give the seed colour as black, Tutin (1952) gives it as dark brown. Observation showed that seed colour varies from light brown to black, sometimes on the same plant.

Professor Tutin has kindly informed me that his original observations on fruit characters were carried out with small samples, and that subsequent sampling showed the characters, as described above, to be inconstant.

All the known colonies of C. × *lucana* were separated by shorter distances from pure colonies of the parent species than from other hybrid colonies, and were thus relatively unlikely to be pollinated by the same taxon (although eight such pollinations were successfully performed artificially). Fruit found on hybrid colonies varied in form from and between that of the two species, and measurements are thus omitted from Table 4, being of little or no significance.

5. DISCUSSION

Since the hybrid is highly fertile and capable of forming backcrosses with either parent, as well as forming F_2 hybrid generations, intermediates of every possible degree may be expected to occur. Fig. 6 shows that the hybrid characters in the area investigated already overlap those of *C. sepium*, but not those of *C. silvatica*. If individual flowers are included it is found that the only *Ts* values possible (4–40) that are not occupied by at least one flower are 37, 39 and 40.

Because of this overlap it is difficult to designate a range of structure in terms of Ts values to the hybrid, but the author considers this range to be 13–21, whilst those of C. sepium and C. silvatica are 6–12 and 23–31 (–35) respectively. In colony 71 (Fig. 5) it is interesting to note that the average Ts value for the hybrid element (17.5) is extremely close to the halfway point (18) between the mean Ts values for C. sepium (9) and C. silvatica (27). The mean Ts values for C. sepium and C. silvatica colonies in colonies 1–70 (Fig. 6) are exactly the same as in colony 71, namely 9 and 27 respectively. The mean value for the hybrid colonies is lower, however, being 16. This indicates that the majority of hybrid colonies encountered resulted from crosses involving smaller than average parents, since from the data in Fig. 5 it seems that hybrids are almost exactly intermediate between their two parents.

The hybrid colonies in the area of study (12 out of 70 colonies) represent some 17% of the total. The name of the hybrid, $C \times lucana$, should theoretically be applied to all plants possessing a genotype derived from two species, whether or not these plants are morphologically distinguishable from either parent.

Undoubtedly, from this situation, originally hybrid populations may merge into either parent by continued backcrossing to that parent. The results of this, hybrid swarms, provide interesting genetical investigations into introgression and its effects. Anderson (1949) quite correctly criticises workers who, having proved that hybrids exist in the wild, do not continue their investigations further along the lines indicated above. Unfortunately, due to lack of time and facilities, genetical investigations could not be pursued in the present situation. Introgression may well have a bearing on pink-flowered forms of C. sepium

and *C. silvatica*, since Brummitt & Heywood (1960) state that the former species may have pink corollas, but that *C. silvatica* is never predominantly pink-flowered. If hybridisation occurs, and back-crossing to *C. silvatica* follows, pink-flower genes will undoubtedly leak into the latter species. Brummitt & Heywood (1960) refer all predominantly pink-flowered plants with inflated bracteoles to their *C. pulchra* (which Scholz (1960) reduces to a variety of *C. sylvestris*). I have so far reached no personal conclusions as to the taxonomic status of pink-flowered Calystegiae.

The present work may at first seem in part open to the same criticism that Pritchard (1960) describes for *Gentianella*. This is that we have (possibly in error) assigned equal importance to each of the four characters used in the hybrid index, since each was transferred to a scale of 1–10. Pritchard, however, was attempting to separate a series of populations, previously recognised as a single group, into two subspecies. The present author suggests that in the present situation this criticism (if it existed) would assume far less importance, since here we are attempting to find a numerical method of expressing the difference between two well-defined taxa, a difference at once obvious to the eye. When we find that method, and have proved it to be successful with a large number of colonies (only plants appearing intermediate to the eye actually falling between the two species ranges), it is relatively much less important than in the case of *Gentianella* that some of the characters may be given slightly too much or too little significance. In the case of *Gentianella* there is no good yardstick informing the worker that he is on the right lines. In addition, the fact that many workers have used the hybrid index with great success (cf. Anderson, 1949) would suggest that its method can be safely applied here.

As has been found with many other self-incompatible plants, the majority of outcrossings prove successful. Assuming the genetical basis of incompatibility in *Calystegia* to be of the normal S-allele type, results would tend to suggest that there are a very large number of S-alleles involved in this system, since the chances of finding a similar S-allele even in fairly close proximity (outside the same clone) are apparently small. It seems likely, therefore, that *C. silvatica* has been introduced into this country on numerous occasions.

As far as the experimental taxonomist is concerned, there is little doubt as to the category that the *C. sepium-silvatica* complex falls into. Using the nomenclature of Turesson, as modified by Clausen, Keck and Hiesey (Heslop-Harrison, 1953), this complex would comprise a single ecospecies if we assume that hybridisation involves no loss of fertility. The number of results so far obtained, however, are not sufficient to be certain of this, and it may be that some slight loss in fertility is incurred, especially after several generations. In this case the complex would fall within a single coenospecies.

Not until Tutin (1959) relegated C. silvatica to C. sepium subsp. silvatica (Kit.) Tutin* had the two taxa been regarded as anything but 'good' species by modern British taxonomists. Brummitt & Heywood (1960) keep the two species apart, mainly on the evidence that they do not hybridise. Although, in fact, they do readily hybridise, and natural hybrids are very widespread, many pairs or more of taxa are known which hybridise freely and yet are retained as species nomenclaturally. One of the best examples is the genus Geum, where whole groups of species comprise a single coenospecies, and several pairs a single ecospecies (Gajewski, 1957). As Gajewski points out, the evolution of an interspecific sterility barrier may not occur apace with morphological differentiation and there is no reason to assume that the two are directly related. A quick glance through the species of *Calystegia* in the British Museum herbarium shows that several of them are quite close to C. sepium and C. silvatica in appearance, and from the diverse remarks made on the sheets it appears that considerable difficulty has been encountered in the past in naming many specimens. At least two or three species are closer to C. sepium than is C. silvatica. It is thus clear that only after a detailed cytotaxonomic investigation, on the lines of Gajewski's work on Geum, will it be possible to state with any certainty the relation of C. sepium and C. silvatica to the other species and to each other. Until this is done it is not possible to assess the importance of interfertility between C. sepium and C. silvatica as

*I have since found that this combination was first made by Braun-Blanquet & Maire, Fl. Maroc, 217 (1924).

taxonomic evidence. If several other groups of species in the genus are found to be capable of hybridisation amongst themselves and/or with the *C. sepium/silvatica* group, then all the species are best treated as taxonomically distinct, since introduction of the sub-species concept here would result in very few species with numerous subspecies – a structure which Gajewski rejects. It might equally be found, on the other hand, that the only two species hybridising in the genus are *C. sepium* and *C. silvatica*, when introduction of the subspecies concept (cf. Tutin, 1959) would be advantageous, especially since *C. silvatica* is severely limited geographically when compared to *C. sepium*. The author suggests, however, that until any such monographic work is undertaken the two taxa, *C. sepium* and *C. silvatica*, are best maintained as distinct species, producing the fertile hybrid $C. \times$ *lucana*.

I do not suggest that conclusive proof of the hybrid origin of the numerous intermediates found in the wild has been produced, but I consider that the circumstantial evidence outlined above is sufficient to assume this. The results are published as they stand, as there seems to be little chance that I shall be able to pursue the subject further for some time.

6. SUMMARY

1. Of the several characters which have been used in the past to separate *C. sepium* and *C. silvatica*, some are considered inconstant, their use being limited to extreme or 'typical' plants. It would seem that the best diagnostic characters are the sizes of some parts and the shape of the bracteoles, so that plants without flowers may not be referable to their correct taxon in all cases.

2. By means of a hybrid index of four variables C. sepium and C. silvatica may be easily separated, and any intermediates (hybrids) which might exist recognised. Of seventy small colonies examined twelve (or 17%) proved to be of hybrid derivation. Two polymorphic colonies were also investigated, one being solely a mixture of the two species, the other a mixture of the two species with the hybrid between them.

3. In the wild, all three taxa are found to be commonly fertile from natural pollination, and pollen from them is seen to be full and spherical, although germination in glucose solution did not occur.

4. Pollination experiments showed that all three taxa are (probably 100%) selfincompatible. Pollen tube studies showed that the contorted tubes resulting from germination on incompatible styles entered the stigma for a short distance, but that they failed to reach anywhere near the style.

5. Further pollination experiments showed that the three taxa are freely interfertile in all six possible combinations, and that small populations are usually single clones, whilst large (polymorphic) ones are multiclonal.

6. All seeds obtained (either from natural pollinations or from artificial intraspecific or interspecific crosses) proved easy to germinate when in the sub-mature stage. After the seeds become completely mature and desiccated germination may be effected by chipping followed by a long period of soaking, but naturally the seeds appear to enter a dormant stage. Thus the presence of full seeds always indicates fertility.

7. Literature research showed that the hybrid has been long known in North Africa and in Italy, as *C. lucana* and *C. barbara*. The hybrid should be called $C. \times lucana$ (Tenore) G. Don. A list of the most important diagnostic measurements separating the three taxa is given. $C. \times lucana$ is known from several areas of England and Wales and also from the Channel Isles, Spain, Italy, Albania, Greece, S.W. Russia and N.W. Africa.

8. Difficulties in assigning limits to a fertile hybrid are pointed out, and the possibilities of introgression and its possible effects on pink-flower characters are commented upon.

9. The present situation is compared to that in *Geum*, and reasons put forward for maintaining the two species distinct, at least for the present.

A specimen of the hybrid from Colony 71 (Ts = 16), on which many of the pollination experiments were performed, has been deposited in the British Museum herbarium (**BM**).

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