

THE ORIGIN OF *CARDAMINE FLEXUOSA* WITH EVIDENCE FROM MORPHOLOGY AND GEOGRAPHICAL DISTRIBUTION

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ABSTRACT

The morphology and geographical distribution of *Cardamine flexuosa* and some closely related species have been examined. The data obtained suggest that *C. flexuosa* arose by allopolyploidy from *C. impatiens* and *C. hirsuta*.

INTRODUCTION

C. flexuosa is a tetraploid which may have arisen by autopolyploidy or allopolyploidy. Species which, on morphological grounds, may have been involved in its origin are: *C. hirsuta* L., *C. impatiens* L., *C. parviflora* L., *C. caldeirarum* Guthnick and *C. trichocarpa* Hochst.

TABLE 1. Chromosome numbers of some *Cardamine* spp. which are related morphologically to *C. flexuosa*.

<i>Species</i>	<i>2n</i>	<i>Reported by:</i>
<i>C. hirsuta</i>	16	} Several authors, see Löve & Löve (1961)
<i>C. impatiens</i>	16	
<i>C. parviflora</i>	16	
<i>var. arenicola</i>	22,24	Smith 1938, Mulligan 1965
<i>C. caldeirarum</i>	?	Rollins 1966
<i>C. trichocarpa</i>	32	Not counted
<i>C. flexuosa</i>	32	Morton 1961 (as <i>C. hirsuta</i>)
		Several authors, see Löve & Löve (1961)

C. hirsuta appears to have been closely concerned in the evolution of *C. flexuosa*. Morphological resemblances are so strong that it is sometimes difficult to distinguish the species, especially when only winter rosettes are available. Indeed, in the Floras of many European countries it is only in the present century that the species have been distinguished, and as recently as 1943 they were recombined (under *C. hirsuta* L.) by Biswas (1943).

The hybrid *Cardamine flexuosa* × *hirsuta* (*C. zahlbrucknerana* O. E. Schulz) is known from the wild. It was reported by Schulz (1903) as occurring naturally in Austria and there are specimens in the collections of the British Museum (BM) from localities in the Basses Pyrénées and Montgomeryshire. These specimens have been overlooked until recently, which may indicate that the hybrid has often not been recognized in the wild. It has been synthesized by Benoit (1957) and on separate occasions by both of the present authors.

C. flexuosa does not at first sight appear to be as closely related to any other species of *Cardamine* as it is to *C. hirsuta*. Several hybrids were reported by Schulz (1903) but only *C. flexuosa* × *hirsuta* and *C. flexuosa* × *pratensis* have been proved by synthesis (Lövkvist 1957). Possible origins of *C. flexuosa* may be summarized as in Table 2.

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TABLE 2. Possible origins of *Cardamine flexuosa*.

ALLOPLOID ORIGIN	
1. <i>Cardamine hirsuta</i> × <i>C. caldeirarum</i>	} (Doubling of chromosome complement $2n = 16 \rightarrow 32$) } CARDAMINE FLEXUOSA
2. <i>Cardamine hirsuta</i> × <i>C. impatiens</i>	
3. <i>Cardamine hirsuta</i> × <i>C. parviflora</i>	
AUTOPLOID ORIGIN	
4. <i>Cardamine hirsuta</i>	} (Doubling of chromosome complement $2n = 16 \rightarrow 32$) } CARDAMINE FLEXUOSA
5. <i>Cardamine parviflora</i>	
6. <i>Cardamine impatiens</i>	

When *C. hirsuta* and *C. flexuosa* are compared they are seen to differ in several characters. If *C. flexuosa* is of hybrid origin between two species then the characters of the unknown parent can be predicted as in Table 3. The characters used are taken from Jones (1964).

TABLE 3. Predicted characters of the other species involved in the origin of *C. flexuosa*.

Character	<i>C. hirsuta</i>	<i>C. flexuosa</i>	Unknown Parent
1. Growth habit	annual	annual and biennial	biennial
2. Height	7-30 cm	10-50 cm	13-70 cm
3. Stem hairs	absent	present at base of stem	present throughout stem
4. Numbers of pairs of leaflets on basal leaves	1-3	3-6	5-9
5. Shape of leaflets	obovate to orbicular	ovate	ovate to elliptic
6. Leaf margin	crenate	crenate to dentate	dentate
7. Number of cauline leaves	2-4	4-10	6-16
8. Number of pairs of leaflets on cauline leaflets	2-5	5+	5-9
9. Number of stamens	6 (4)	6	6
10. Siliqua size	18-25 × 1 mm	12-25 × 1 mm	12-25 × 1 mm
11. Style size	0.5-1.0 mm	1-1.5 mm	1.5-2.0 mm
12. Diploid chromosome number	16	32	16
13. Seed size	0.9-1.0 × 0.7-0.8 mm	1.0-1.2 × 0.8-0.9 mm	1.1-1.4 × 0.9-1.0 mm

The predicted characters of the putative parent are compared with the characters of *C. caldeirarum*, *C. impatiens* and *C. parviflora* in Table 4.

Banach (1950) suggested that *C. flexuosa* might be an autotetraploid derivative of *C. hirsuta*. This suggestion was made because of the close morphological resemblance between the species. The assumption made (that an autotetraploid derivative closely resembles the diploid) appears to be supported by evidence from other plant species, but here we are dealing with a diploid and a tetraploid which differ in a significant number of features, and this invalidates Banach's conclusion. In fact, as will be clear from the data given later in the paper, the synthesized autotetraploid resembles diploid *C. hirsuta* more closely than *C. flexuosa* and bears out this point. According to Stebbins (1957) there is a correlation between autopolyploidy and outbreeding, and between allopolyploidy and inbreeding in all published examples. It is worth noting that *C. flexuosa* is inbreeding; if Stebbins' correlation holds in this case, we would expect the plant to be an allopolyploid.

TABLE 4. Comparison of the characters of possible parent species with those predicted from a comparison of *C. hirsuta* and *C. flexuosa*.

Character (as Table 3)	<i>C. caldeirarum</i>	<i>C. impatiens</i>	<i>C. parviflora</i>
1	2	1	0
2	1	2	0
3	1	0	0
4	1	0	2
5	1	2	2
6	1	2	0
7	—	—	—
8	1	2	2
9	2	2	2
10	0	1	2
11	—	—	—
12	—	2	2
13	—	2	0
Total	10	16	12
Total possible	18	22	22
% affinity	55%	72%	54%

Explanation of scoring

2 = corresponds to predicted character.

1 = \pm corresponds to predicted character.

0 = does not correspond to predicted character.

$$\% \text{ affinity} = \frac{\text{Actual score}}{\text{Total possible}}$$

The morphology of *C. flexuosa* and *C. hirsuta*, introduced into New Zealand, has been studied by Pritchard (1955, 1957) who reports differences between the species similar to those seen in European material.

Experimental investigation was confined to *C. flexuosa*, *C. hirsuta*, *C. hirsuta* auto-tetraploid, the F_1 hybrid *C. flexuosa* \times *hirsuta* and *C. impatiens*. All observations have been made on plants cultivated under similar conditions.

LEAF MORPHOLOGY

C. flexuosa and some apparently closely related species show leaf polymorphism. For comparison of leaf characters, samples were taken from the rosette and from the lower and upper parts of the flowering stem. In *C. flexuosa* and *C. impatiens* these three categories of leaf could be easily distinguished. In *C. hirsuta*, some samples showed three main leaf types, but in others it was possible to distinguish only rosette and cauline leaves.

In the synthesized F_1 hybrid between *C. flexuosa* and *C. hirsuta*, only small differences were observed between the lower and upper cauline leaves.

The characters which were analysed statistically are: leaf length, number of pinnæ per leaf and the dimensions of the terminal leaflet. The means of these characters are summarized in Tables 5–7. The number of measurements made in each sample was twenty except for the *C. hirsuta* autotetraploid where the extremes of the ranges of variation were estimated. More precise estimates cannot be given because each tetraploid plant possesses comparatively few leaves.

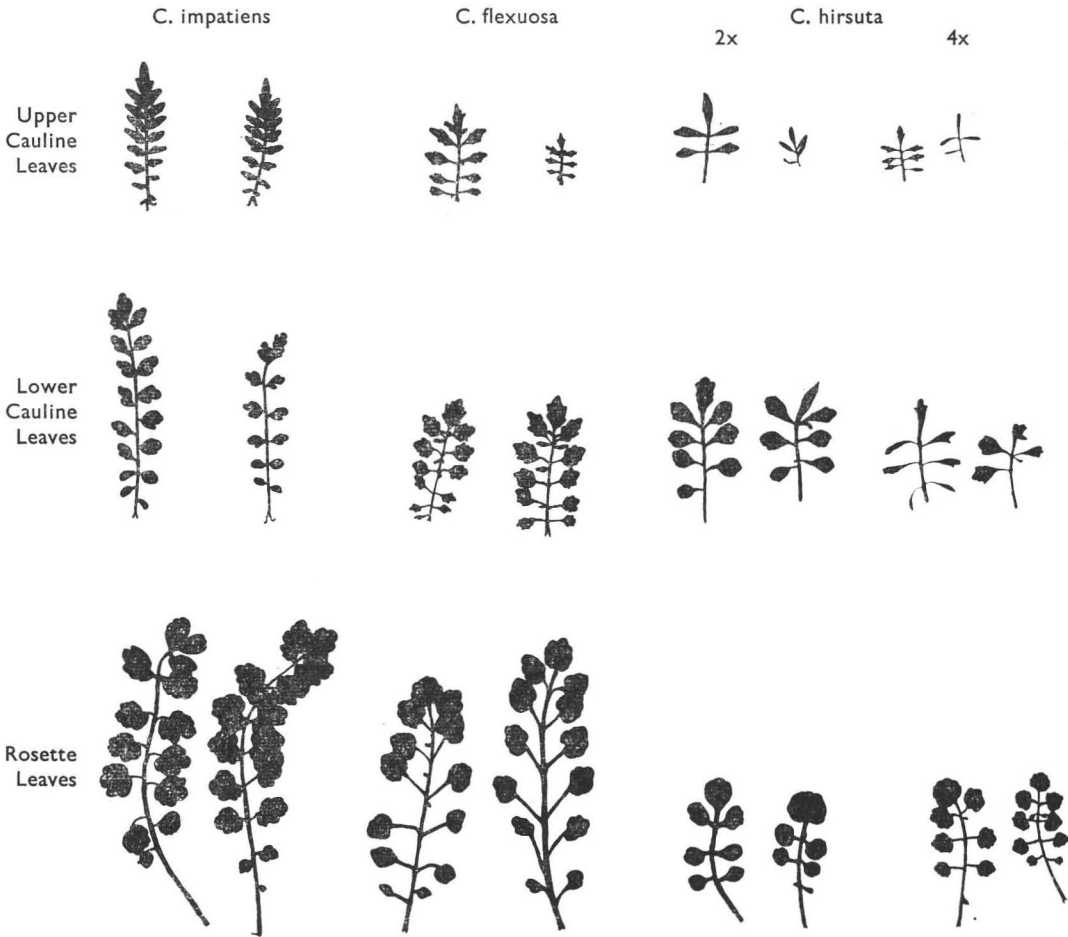


Fig. 1. Leaf morphology of *Cardamine impatiens*, *C. flexuosa* and *C. hirsuta*.

Fig. 2.

	<i>C. impatiens</i>		<i>C. hirsuta</i>				<i>C. flexuosa</i>				<i>C. hirsuta</i> x <i>C. flexuosa</i>		<i>C. hirsuta</i>
	S70	S76 S27	Lip	P2	P16	6647	6609	Lym	F1	4x			
<i>C. impatiens</i> S70	0	4	0	1	3	3	3	3	1	2			
<i>C. hirsuta</i>	S76	5	6	7	3	2	5	4	3	5			
	S27	10.4	4	7	7	6	6	6	4	5			
	Lip		48.6	6	2	6	5	5	4	8			
	P2				1	2	1	1	2	8			
<i>C. flexuosa</i>	P16					5	8	7	2	0			
	6647	25.0					11	10	6	5			
	6609		22.5					10	5	4			
	Lym					70.0			6	3			
<i>C. flexuosa</i> x <i>C. hirsuta</i>	F1	8.3		27.0			39.6			2			
<i>C. hirsuta</i>	4x	16.6		54.0			25.0		16.6				

TABLE 5. Dimensions of rosette leaves. N = 20 for leaf measurements.

<i>Species</i>	<i>Mean</i>	<i>Standard deviation</i>	<i>Range in sample</i>
(i) Leaf length (cm)			
<i>C. impatiens</i> S70	19.11	4.30	11.70-23.20
<i>C. hirsuta</i> S76	6.45	0.68	4.30- 9.70
„ S27	7.42	1.50	4.90-11.00
„ P2	4.20	0.72	3.50- 6.30
„ Lip.	5.41	1.45	4.11- 9.20
<i>C. flexuosa</i> P16	15.70	2.60	10.00-19.40
„ 6647	12.91	2.22	7.80-16.60
„ 6609	13.36	2.31	7.15-17.70
„ Lym.	9.93	2.10	5.70-14.10
<i>C. hirsuta</i> × <i>flexuosa</i> F ₁	9.25	1.71	7.75-14.50
4x <i>C. hirsuta</i>	—	—	5.00- 5.80
(ii) Pinnae per leaf			
<i>C. impatiens</i> S70	14.55	1.62	13-17
<i>C. hirsuta</i> S76	6.10	1.16	3- 7
„ S27	7.37	1.09	3- 9
„ P2	6.50	1.90	3- 7
„ Lip.	6.40	1.67	3- 7
<i>C. flexuosa</i> P16	12.70	1.71	11-15
„ 6647	10.82	2.48	9-15
„ 6609	12.25	2.13	11-15
„ Lym.	10.00	1.00	8-13
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	9.25	1.71	5-11
4x <i>C. hirsuta</i>	—	—	7- 8
(iii) Length of terminal leaflet (mm)			
<i>C. impatiens</i> S70	19.40	5.15	10.50-27.00
<i>C. hirsuta</i> S76	7.77	1.81	5.50-13.00
„ S27	13.10	2.48	9.00-18.00
„ P2	6.70	0.90	3.50- 9.80
„ Lip.	7.6	1.13	5.30-10.20
<i>C. flexuosa</i> P16	17.27	2.19	14.50-20.0
„ 6647	12.55	1.99	10.50-16.50
„ 6609	11.33	3.71	9.00-24.00
„ Lym.	12.00	2.20	11.80-16.45
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	11.62	1.93	8.00-13.50
4x <i>C. hirsuta</i>	—	—	6.00-10.00
(iv) Breadth of terminal leaflet (mm)			
<i>C. impatiens</i> S70	13.49	3.94	10.00-19.00
<i>C. hirsuta</i> S76	9.37	1.62	7.00-13.00
„ S27	16.10	3.53	8.00-18.50
„ P2	9.47	2.00	7.50-15.20
„ Lip.	7.70	1.80	5.70-14.60
<i>C. flexuosa</i> P16	16.22	2.27	12.00-22.00
„ 6647	12.85	3.00	8.50-21.00
„ 6609	15.55	2.71	10.00-21.00
„ Lym.	12.00	1.40	11.50-16.50
<i>C. hirsuta</i> × <i>flexuosa</i> F ₁	9.55	1.87	7.00-13.00
4x <i>C. hirsuta</i>	—	—	8.00-11.00

TABLE 6. Dimensions of lower cauline leaves. N = 20 for leaf measurements.

<i>Species</i>	<i>Mean</i>	<i>Standard deviation</i>	<i>Range in sample</i>
(i) Leaf length (cm)			
<i>C. impatiens</i> S70	10.59	2.60	7.30-14.00
<i>C. hirsuta</i> S27	6.50	1.35	3.60- 7.20
„ P2	4.20	0.77	2.85- 5.20
<i>C. flexuosa</i> P16	7.14	1.51	4.50- 9.90
„ 6647	3.94	1.69	3.30- 6.25
„ 6609	4.02	1.40	2.52- 7.20
„ Lym.	6.56	3.40	3.16-10.36
4x <i>C. hirsuta</i>	—	—	3.2 - 4.00
(ii) Pinnae per leaf			
<i>C. impatiens</i> S70	14.12	1.12	13-16
<i>C. hirsuta</i> S27	6.77	1.09	5- 8
„ P2	5.90	0.99	5- 7
<i>C. flexuosa</i> P16	10.50	1.46	7-13
„ 6647	10.45	1.29	9-13
„ 6609	10.21	0.83	9-13
„ Lym.	9.00	1.41	6-12
4x <i>C. hirsuta</i>	—	—	6- 4
(iii) Length of terminal leaflet (mm)			
<i>C. impatiens</i> S70	13.00	2.32	10.00-16.50
<i>C. hirsuta</i> S27	19.00	3.65	13.50-23.50
„ P2	7.90	1.80	6.10-11.50
<i>C. flexuosa</i> P16	15.67	4.58	11.00-22.00
„ 6647	8.16	2.59	4.50-12.00
„ 6609	11.33	2.64	7.83-16.33
„ Lym.	14.20	1.53	11.20-18.70
4x <i>C. hirsuta</i>	—	—	8.00-12.00
(iv) Breadth of terminal leaflet (mm)			
<i>C. impatiens</i> S70	6.50	0.92	5.00- 8.00
<i>C. hirsuta</i> S27	7.27	1.65	5.50-10.00
„ P2	7.40	0.99	5.40-10.13
<i>C. flexuosa</i> P16	11.25	2.81	7.00-15.50
„ 6647	7.09	2.50	4.50-12.00
„ 6609	9.42	1.89	6.21-15.82
„ Lym.	9.90	1.23	7.40-13.60
4x <i>C. hirsuta</i>	—	—	4.00- 6.00

These characters can be divided into those which are the same in two or more samples, indicating affinity, and those significantly different, indicating distinctness. Fig. 2 indicates: (i) the number of characters common to any two of the samples studied, and (ii) percentage affinity between the taxa studied.

There are distinct differences between *C. flexuosa*, *C. hirsuta* and *C. impatiens* in six of the twelve leaf characters studied. These are leaf length and number of pinnae per leaf for rosette, lower cauline and upper cauline leaves. In all these characters *C. flexuosa* is intermediate between *C. hirsuta* and *C. impatiens*.

Stomatal guard cell sizes were measured on rosette leaf terminal leaflets obtained from herbarium specimens. Guard cell length and breadth were measured by means of a camera lucida. The mean sizes are summarized in Fig. 3.

TABLE 7. Dimensions of upper cauline leaves. N = 20 for leaf measurements.

<i>Species</i>	<i>Mean</i>	<i>Standard deviation</i>	<i>Range in sample</i>
(i) Leaf length (cm)			
<i>C. impatiens</i> S70	6.43	1.30	4.15- 8.00
<i>C. hirsuta</i> S76	3.07	0.61	1.45- 4.80
„ S27	3.60	1.10	2.40- 5.20
„ P2	3.30	1.48	1.80- 3.67
„ Lip.	1.98	0.75	1.25- 4.10
<i>C. flexuosa</i> P16	3.26	1.00	2.10- 5.70
„ 6647	2.86	1.86	1.60- 8.70
„ 6609	4.02	1.40	2.00- 8.30
„ Lym.	3.44	1.52	2.05- 7.90
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	3.28	0.82	2.10- 4.70
4x <i>C. hirsuta</i>	—	—	1.80- 2.20
(ii) Pinnae per leaf			
<i>C. impatiens</i> S70	15.10	2.09	11-17
<i>C. hirsuta</i> S76	4.50	0.84	3- 6
„ S27	6.50	3.33	5- 7
„ P2	5.28	0.71	4- 7
„ Lip.	6.60	0.84	5- 7
<i>C. flexuosa</i> P16	7.75	1.16	5- 9
„ 6647	8.13	1.54	8-13
„ 6609	8.11	1.42	5- 9
„ Lym.	7.40	0.84	5- 9
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	7.85	1.08	7-10
4x <i>C. hirsuta</i>	—	—	4- 7
(iii) Length of terminal leaflet (mm)			
<i>C. impatiens</i> S70	18.30	3.78	12.50-22.00
<i>C. hirsuta</i> S76	10.20	3.01	5.50-15.50
„ S27	14.83	3.66	10.50-21.00
„ P2	9.20	0.50	8.00-10.70
„ Lip.	7.15	0.29	6.50- 8.20
<i>C. flexuosa</i> P16	12.00	3.24	8.00-18.00
„ 6647	9.59	4.05	5.00-19.50
„ 6609	11.33	2.64	7.00-11.00
„ Lym.	10.60	1.19	7.30-13.80
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	8.07	2.81	4.00-15.00
4x <i>C. hirsuta</i>	—	—	7.00- 9.00
(iv) Breadth of terminal leaflet (mm)			
<i>C. impatiens</i> S70	6.64	1.04	6.00- 9.00
<i>C. hirsuta</i> S76	4.15	1.59	2.00- 7.00
„ S27	2.16	0.81	1.00- 3.00
„ P2	4.19	0.45	3.25- 6.02
„ Lip.	2.57	0.19	1.80- 3.24
<i>C. flexuosa</i> P16	5.83	1.49	3.00-10.00
„ 6647	6.68	2.00	2.00-12.50
„ 6609	5.55	1.84	3.00- 5.50
„ Lym.	5.80	0.23	5.20- 6.70
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	5.20	0.66	2.50- 8.50
4x <i>C. hirsuta</i>	—	—	1.50- 2.50

FLORAL MORPHOLOGY

The floral morphology of *C. flexuosa*, *C. hirsuta*, *C. impatiens* and the F₁ hybrid *C. flexuosa* × *hirsuta* was examined. The numbers of floral parts observed were usually sepals four, petals four, stamens six, and ovary one, but in diploid *C. hirsuta*, individuals

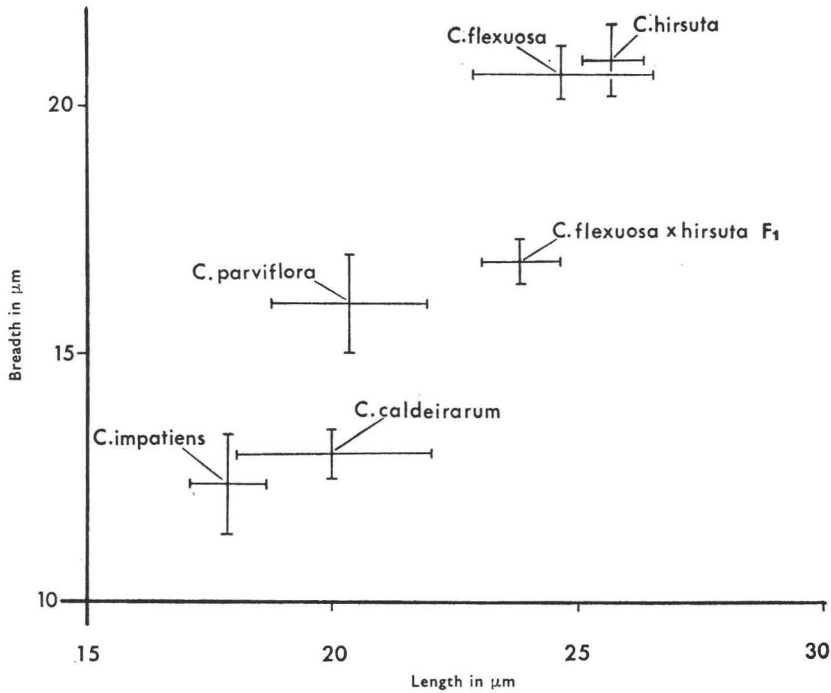


Fig. 3. Sizes of stomata, means \pm twice standard error of the mean.

with only four stamens were occasionally observed, while in autotetraploid *C. hirsuta*, four stamens were consistently observed. One of eleven collections of *C. impatiens* studied was apetalous.

Measurements of sepals, petals and stamens were made on flowers which had opened and which possessed stigmas capable of pollination, by photographing the dissected flower parts and measuring images produced by projecting the negative. This was judged by observing stigmatic papillae which on young stigmas have not developed and which on older stigmas wither. Between these stages, stigmatic papillae are prominent for a period of about two days and experimental pollination is possible. In the Cruciferae, the six stamens are normally arranged in two pairs of long and one pair of short stamens. The longer and shorter stamens were separated for analysis.

C. flexuosa was found to be intermediate in sepal size but larger than *C. impatiens* and *C. hirsuta* in petal and stamen size. When compared with autotetraploid *C. hirsuta*, *C. flexuosa* was seen to have smaller sepals and anthers while the petals were the same size. Autotetraploid *C. hirsuta* had larger floral parts than the diploid.

Differences observed between the shape of the stigma in the mature fruit in *C. hirsuta*, *C. impatiens* and *C. flexuosa* are summarized in Fig. 4.

POLLEN SIZE

Pollen shape was found to vary between ellipsoidal and spherical depending on the degree of dehydration, when examined without mountant, or with concentration of the mountant, e.g. glycerine. Changes of size were noted at the same time as the changes of shape. For these reasons all measurements were made on pollen mounted in acetocarmine in 45 per cent. acetic acid. The diameters of the pollen samples measured are summarized in Table 8. Mean pollen volume was calculated from half the mean diameter for each sample.

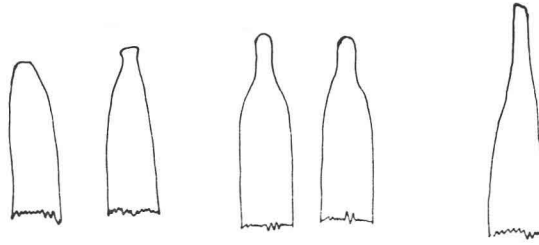
Fig. 4. Stigma shape in mature pod; (left to right) *C. hirsuta*, *C. flexuosa*, *C. impatiens*.

TABLE 8. Pollen diameters.

Species	Sample reference	Number in sample	Mean diameter in μm	$2 \times$ Standard error of mean	Pollen volume ($\times 10^3 \mu\text{m}^3$)
<i>C. flexuosa</i>	Lymington	500	23.58	0.18	6.86
	P30	400	29.33	0.15	13.21
	Coimbra	300	25.07	0.82	8.25
<i>C. flexuosa</i> (male sterile)	6609	260	20.21	1.04	4.32
<i>C. impatiens</i>	S70	412	18.83	0.19	3.50
<i>C. hirsuta</i>	S27	104	29.20	1.66	13.04
	Frome	201	23.86	0.62	7.11
Synthetic <i>C. hirsuta</i> \times <i>flexuosa</i>	F ₁ hybrid	108	21.90	1.84	5.50
	F ₂ hybrid	100	21.76	0.91	5.39
<i>C. hirsuta</i> (4x), synthetic autotetraploids	S27C3	180	31.60	0.28	16.52
	S27C23	64	36.09	0.99	24.61
	S27C6	278	40.43	1.10	34.60

GEOGRAPHICAL DISTRIBUTION

The geographical ranges of these species cited by Schulz (1903) in his monograph of *Cardamine* indicate that the most widespread species have well differentiated regional morphological types; some of these are treated as species in more recent accounts (e.g. Hultén 1958). *C. hirsuta* is commonly anthropochorous. It seems that the very widespread *C. flexuosa* is not of recent origin, and from their present distributions *C. caldeirarum* and *C. trichocarpa* are unlikely to have been involved in its origin.

TABLE 9

Geographical distribution of *C. flexuosa* and related species.

Species	Distribution
<i>C. flexuosa</i> subsp. <i>flexuosa</i>	Europe, Asia Minor and possibly native in Eastern N. America.*
<i>C. hirsuta</i> subsp. <i>hirsuta</i>	Cosmopolitan in the north temperate zone and widespread in the tropics and southern hemisphere.** (The species is a successful anthropochore whose region of origin is uncertain.)
<i>C. impatiens</i>	Europe; W. & C. Asia.
<i>C. parviflora</i> subsp. <i>parviflora</i>	Europe; Algeria; Iran; Siberia; China.***
<i>C. caldeirarum</i>	Açores.
<i>C. trichocarpa</i>	E. African mountains; Ceylon; Assam.

* Other subspecies and allied species in E. Asia and N. America.

** Other subspecies in N. America and E. Asia.

*** Subsp. *virginica* in N. America.

Hultén (1950) in his classification of Scandinavian plants into migrational groups and distribution types considers *C. flexuosa*, *C. hirsuta*, and *C. impatiens* to be in the same migrational group—that of southern plants adapted to conditions of north-west Europe.

His maps show that *C. impatiens* and *C. hirsuta* do not have such a widespread or northerly distribution as *C. flexuosa* in Scandinavia. *C. impatiens* is primarily a species of moist woodlands and *C. hirsuta* of open, drier habitats; *C. flexuosa* is ecologically diverse, and occupies a wide range of intermediate habitats.

DISCUSSION

The impression that *C. flexuosa* and *C. impatiens* have morphological affinities has been confirmed by closer examination. However the lack of data concerning *C. parviflora* and *C. caldeirarum* does not allow the relationship of these species to *C. flexuosa* to be fully established. The chromosome number of *C. caldeirarum* has never been determined. The mean stomatal guard cell size of this species and *C. parviflora* is close to *C. impatiens*, indicating the possibility that they are diploids (see Fig. 3). The pollen volume of *C. caldeirarum* is similar to that of *C. hirsuta*. *C. caldeirarum* is morphologically similar to *C. flexuosa* and if it is a diploid the possibility that *C. flexuosa* is an autotetraploid derivative of it cannot be excluded on the present evidence. The morphology of *C. impatiens* and *C. parviflora* seems to be sufficiently distinct from *C. flexuosa* to preclude the possibility that they may have given rise to it by autotetraploidy.

Comparison of the leaf morphology of *C. flexuosa* with *C. hirsuta* and *C. impatiens* shows that the affinity of *C. flexuosa* with either species is of the same order. In both cases about 25 per cent. of the characters of *C. flexuosa* are shared with *C. hirsuta* and *C. impatiens*. Autotetraploid *C. hirsuta* and the F_1 hybrid *C. flexuosa* \times *hirsuta*, on the other hand, show a greater affinity with one of the species or the other. Autotetraploid *C. hirsuta* shares about 50 per cent. of characters with *C. flexuosa*. *C. flexuosa* \times *hirsuta* F_1 shares about 40 per cent. of characters with *C. flexuosa* but only 27 per cent. with *C. hirsuta*. These facts are in accordance with the hypothesis that *C. flexuosa* is not autotetraploid *C. hirsuta*, but is intermediate between *C. hirsuta* and *C. impatiens*, and arose by allopolyploidy. This is further supported by the fact that, in the characters which show statistically significant differences, *C. flexuosa* is intermediate between *C. hirsuta* and *C. impatiens*.

Floral morphology varies less than leaf morphology and may be related to the prevalence of inbreeding. This contrasts with the diversity of flower size, shape and colour seen in the outbreeding *Cardamine pratensis*. *C. hirsuta*, *C. flexuosa* and *C. flexuosa* \times *hirsuta* F_1 shared five of the six flower characters studied. *C. flexuosa* and autotetraploid *C. hirsuta*, and *C. impatiens* and *C. hirsuta* (both diploid and autotetraploid) shared three characters. In contrast, the shape of stigmas from mature pods varies considerably (see Fig. 4) and *C. flexuosa* is again seen to be intermediate between *C. hirsuta* and *C. impatiens*.

Apart from the characters examined statistically, the general impression gained is that, under the conditions of experimental cultivation, *C. flexuosa* is morphologically intermediate between *C. hirsuta* and *C. impatiens*. This is so for example in growth habit, where *C. hirsuta* is short and upright and *C. impatiens* tall and lax, and in the features of the margin of the leaflets (see Fig. 1). It is also the case in occurrence of stipules, which are present in *C. impatiens*, absent from *C. hirsuta* and 'vestigial' in *C. flexuosa*. These differences are not so apparent in herbarium material, or in plants in the wild.

The pollen-volumes of the plants examined present an interesting problem. The synthetic autotetraploid of *C. hirsuta* has pollen twice the volume of diploid *C. hirsuta*. However, the wild tetraploid *C. flexuosa* has pollen of a similar volume to that of *C. hirsuta*. This is circumstantial evidence which supports the hypothesis, based upon external morphology, that *C. flexuosa* did not arise as an autotetraploid of *C. hirsuta*. A possible explanation may lie in the low pollen volume of *C. impatiens*, the other putative diploid parent of *C. flexuosa*: although *C. impatiens* and *C. hirsuta* both have $2n = 16$, the pollen-volume of the former is half that of the latter species. A hybrid between these two might be expected to have pollen of an intermediate size, and the 'raw' allopolyploid derivative

should have pollen somewhat larger than that of *C. hirsuta* (Table 10). The observation of pollen volumes for *C. flexuosa* rather lower than expected on the assumption that tetraploidy doubles the pollen-volume, may be due to intergenomic segregation of genetic factors determining pollen size and to selection subsequently favouring small microspores. *Nasturtium officinale* sensu lato presents a parallel case. *N. microphyllum* is an allo-octoploid, one of whose parents is the tetraploid *N. officinale* sensu stricto (Howard & Manton 1946).

TABLE 10. Comparison of observed and predicted pollen volumes.

<i>Species</i>	<i>Genotype</i>	<i>Predicted pollen volume</i> $\times 10^3 \mu\text{m}^3$	<i>Observed means of pollen volume</i> $\times 10^3 \mu\text{m}^3$
(i) <i>Diploids</i>			
<i>C. impatiens</i>	AA	—	3.5
<i>C. hirsuta</i>	BB	—	7.1-13.0
<i>C. impatiens</i> \times <i>hirsuta</i> F ₁	AB	5.3-8.2	—
(ii) <i>Triploids</i>			
<i>C. flexuosa</i> \times <i>hirsuta</i> F ₁	ABB	6.2-10.6	5.5
(iii) <i>Tetraploids</i>			
Tetraploid	AAAA	7.0	—
derivatives of above diploids	BBBB	14.2-26.0	16.5-34.6
	AABB	10.6-16.5	—
<i>C. flexuosa</i>	AAAA AAAB AAAB	* } 7.0-16.5	6.9-13.2

* = hypothetical genotypes, refer to text for explanation.

The pollen volumes for the two species, calculated from the data of Green (1955), are $8.2-14.2 \times 10^3 \mu^3$ and $4.1-9.2 \times 10^3 \mu^3$ respectively. Here also the upper limits of the range of the allopolyploid pollen volumes is rather less than might be expected from a doubling of the tetraploid pollen volume. The same explanation may be given for the fact that the stomatal guard-cells of *C. hirsuta* and *C. flexuosa* are statistically indistinguishable in size (Fig. 3).

As there are taxonomic difficulties in these species, the reported geographical distributions do not allow reliable conclusions to be drawn about the 'centre of origin' of the group. However, the distribution of the species in Scandinavia is significant; there is a difference between the more southerly and restricted diploids and the tetraploid which is more widespread and extends farther north. *C. hirsuta* and *C. flexuosa* show a similar and widespread distribution in the British Isles (Perring & Walters 1962). *C. impatiens* is rarer and restricted to a narrower range of habitats, mainly on limestone.

The differences in the relative distribution of the diploid and tetraploid species in Scandinavia and Britain may exist for several reasons. The glaciation of Scandinavia was more extreme than in Britain and present day climates are more severe in Scandinavia. Differences in distribution may therefore reflect differences in genetic variability and hence adaptability, i.e. ability to invade new habitats and to diversify genetically in developing vegetation types. This makes the allopolyploid origin for *C. flexuosa* more likely than an autopolyploid origin whose long-term consequence would be the stabilization of existing genotypes.

CONCLUSIONS

C. flexuosa is morphologically distinguishable from both diploid and autotetraploid *C. hirsuta*, and it does not appear likely to have arisen from *C. hirsuta* by autopolyploidy. Lack of evidence does not allow comment on the possibility of autotetraploid origin from

C. caldeirarum. At the same time the available evidence concerning the morphology of *C. hirsuta* and *C. impatiens* suggests that *C. flexuosa* may have arisen by hybridization of these diploid species followed by doubling of the chromosome complement.

The similarity in pollen volume and stomatal guard cell size between the tetraploid *C. flexuosa* and the diploid *C. hirsuta* is most easily explained by the origin of the tetraploid species from the hybridization of *C. hirsuta* with another diploid species characterized by smaller guard cells and microspores; *C. impatiens* has such features.

The geographical distribution of the species in Scandinavia also supports this hypothesis: the tetraploid has a wider ecological amplitude than either of its presumed parents, but the habitats lie, in most respects, between the extremes of the putative parent species.

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APPENDIX

Sources of material. (Collections of wild origin, except where indicated)

<i>C. impatiens</i> S70	Berlin Botanic Garden, Germany.
<i>C. hirsuta</i> S76	Yalta Botanic Garden, U.S.S.R.
„ S27	Palermo Botanic Garden, Sicily.
„ P2	Wisley, England.
„ Frome	Frome, England.
„ Lip.	Liphook, England.
<i>C. flexuosa</i> P16	Denmead, nr. Portsmouth, England.
„ Coimbra	Coimbra Botanic Garden, Portugal.
„ 6647	Lac du Gaube, Hautes Pyrénées, France.
„ 6609	L'Hospitalet, Pyrénées Orientales, France.
„ Lym.	Lymington, England.
„ P30	Lock's Bridge, Sussex, England.
<i>C. flexuosa</i> × <i>hirsuta</i> F ₁	Experimentally produced hybrid between <i>C. flexuosa</i> P16 and <i>C. hirsuta</i> S76.
<i>C. hirsuta</i> 4x S273	} Experimentally induced autotetraploids from <i>C. hirsuta</i> S27.
„ S276	
„ S2723	