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From the Department of Botany, University of Reading

**Cytotaxonomy and Breeding System of the Genus *Biscutella*
(*Cruciferae*)**

By

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Key Words: Angiosperms, *Cruciferae*, *Brassicaceae*, *Biscutella variegata* complex, *B. laevigata* complex. —Chromosome numbers, variation, aneuploidy and polyploidy, chromosomal imbalance, protogyny, inbreeders and outbreeders.

Abstract: Chromosome counts were determined for 46 populations of *Biscutella* representing 28 taxa. The genus was found to contain diploid taxa with $2n = 12, 16$ and 18 , tetraploid taxa with $2n = 36$ and hexaploid taxa having $2n = 54$. *B. laevigata* L. s. l. consists of diploid and tetraploid populations which are poorly differentiated morphologically. Tetraploid *B. laevigata* s. l. and hexaploid *B. variegata* BOISS. & REUTER (s. l.) are characterized by chromosomal instability. The variation in chromosome numbers and the occurrence of polyploidy is discussed in relation to the taxonomy of the genus. An investigation of the breeding system showed that most of the annual species were self-compatible and partly inbreeding and most of the perennial species self-incompatible and, therefore, outbreeding, while one annual species, *B. cichoriifolia* LOISEL., showed both systems.

The genus *Biscutella* L. (*Cruciferae*) is a genus of some 40 species and easily recognisable by its highly distinctive didymous fruits, resembling a pair of spectacles. Except for *B. laevigata* L. s. l. which extends into central Europe, most of the species are found in the Mediterranean region. The genus is notorious for its taxonomic difficulty and the two available revisions, by MALINOWSKI (1910) and by MACHATSCHKI-LAURICH (1926), differ widely in their approach — the former adopting a broad species concept, and the latter a narrow one. The difficulties are caused by the relative uniformity of the floral and fruiting characters, and the unreliability of those features that do vary, coupled with the reliance on

vegetative features, especially leaf-shape to separate species and subspecies, despite a lack of knowledge of the range of plasticity of such features. It has been pointed out that leaf-shape can be modified by different growth conditions and the time of initiation of flowering (HEYWOOD 1964) and detailed developmental studies are needed.

As a consequence, although the species usually have a characteristic and recognizable appearance ("facies"), they are difficult to describe precisely and intermediate variants frequently occur. A particular area of taxonomic complexity is the *B. laevigata* complex. This was divided by MACHATSCHKI-LAURICH (1926) into a large number of subspecies and varieties. Subsequently MANTON (1932, 1934, 1937) undertook an extensive series of cytological and crossing studies in *Biscutella*, especially in *B. laevigata* s.l. which she showed to consist of a series of diploid and tetraploid populations, corresponding quite well with MACHATSCHKI-LAURICH's taxonomic/phylogenetic arrangement.

The tetraploids were found by MANTON to be plants of high altitudes, forming a more or less continuous area of distribution in the Alps and mountains of South and South-Eastern Europe. The diploids, on the other hand, were found at low altitudes and had a much more restricted and discontinuous distribution ranging from France to the Balkan peninsula. MANTON regarded the diploids as glacial (or pre-glacial) relicts, occurring in areas that were not covered by the ice sheet in the Pleistocene glaciation while the tetraploids she regarded as derived populations which migrated from other localities into their present area which was glaciated until quite recently.

In effect, MANTON further suggested that the diploid and tetraploid cytotypes be each regarded as separate despite their weak morphological differentiation.

Subsequent cytological studies on the genus have been surprisingly few, and with the exception of those by SCHÖNFELDER (1968) have mainly consisted of counts for one or two species. Details may be obtained from the literature. This present study is the first time that a comprehensive cytotaxonomic survey of the genus has been attempted. The field collections of the senior author furnished the much needed material for cultivation and chromosome determination. Particular attention was paid to Spanish populations of *Biscutella* which had been neglected by MANTON and most subsequent authors.

Taxonomic studies by HEYWOOD (1964) and by GUINEA & HEYWOOD (1964) on a wide range of Spanish material have shown that *B. laevigata*, especially subsp. *laevigata*, has a much more westerly extension than either MACHATSCHKI-LAURICH, MANTON or more recently MEUSEL (1965) indicated, and in fact it extends across France over the Pyrenees into Spain and Portugal. Plants that closely resemble *B. laevigata* subsp.

laevigata occur in the Cantabrian cordillera and elsewhere in north Spain and a few plants reach the Central Sierras and even further south. Many older records of *B. laevigata* from Spain refer to *B. variegata* BOISS. & REUTER s.l. or *B. sempervirens* L. for which few reliable chromosome counts have been published. It is quite evident that the scheme presented by MANTON was only part of a wider picture which it was hoped that the present study would help to complete.

Since previous knowledge of the genus has been limited largely to taxonomic and cytological studies and little is known about its general biology, this investigation has included some biosystematic components. Self-compatibility and self-incompatibility tests have been performed to determine the extent of inbreeding and outbreeding within the genus. It was envisaged that this might shed some light on the presence of the considerable morphological variation observed within certain species or species groups in the genus.

Materials and Methods

All plants investigated were raised in the University of Reading Plant Science Laboratories greenhouses and experimental grounds from seeds collected in the wild, mainly by J. D. OLOWOKUDEJO, and those of known wild origin received from other collectors and botanic gardens. Voucher specimens are deposited in the Herbarium of the Department of Botany, University of Reading (RNG). The complete list of plants studied, together with their chromosome numbers and localities is given in Table 1.

Chromosome Studies: Squashes were prepared from actively growing root tips pre-treated in a saturated solution of para-dichlorobenzene for 3–4 hours at room temperature. The root tips were then fixed in 3:1 acetic alcohol for 12 hours. They were then hydrolysed in 1 N HCl in a water bath at 60 °C for 6–8 minutes. After rinsing in water the root tips were then stained in propionic orcein for 3 minutes before, and one minute after squashing. Preparations were photographed and made permanent using “Arceton 12” (CCl₂F₂) spray.

Self-Incompatibility Test: Two inflorescence branches containing 5–15 fully formed buds were bagged shortly before anthesis, on two fully grown plants of each taxon. Nylon netting, supported by wire frames, was used for the bagging. A wad of cotton wool was wrapped around each peduncle to prevent damage at the site where the net was fastened. At anthesis pollinations were carried out by hand-selfing flowers of the same inflorescence. Seedset or lack of it was used a criterion of whether the plants were self-compatible or not.

Results

Chromosome Numbers. The results of this investigation are summarised in Tables 1, 2 and 3. Chromosome counts have been determined for 28 taxa, of which those for twelve taxa have not been previously reported. In this genus somatic chromosome numbers form

Table 1. Chromosome numbers and origins of *Biscutella* populations

Species	Collector/source and reference number
<i>B. variegata</i> BOISS. & REUTER	OLOWOKUDEJO 60
var. <i>variegata</i>	OLOWOKUDEJO 62
	OLOWOKUDEJO 88
<i>B. megacarpaea</i> BOISS. & REUTER	OLOWOKUDEJO 92
<i>B. foliosa</i> MACH.-LAUR.	OLOWOKUDEJO 93
<i>B. laevigata</i> L.	BG 780349
subsp. <i>laevigata</i>	BG 780416
	BG 187
	Jardin Bot. 780348
	Genève
	BG 780423
subsp. <i>lucida</i> (DC.) MACH.-LAUR.	Jardin Bot. 780420
	Neuchâtel
subsp. <i>kernerii</i> MACH.-LAUR.	BG 780544
subsp. <i>gracilis</i> MACH.-LAUR.	BG 780311
<i>B. frutescens</i> COSSON	OLOWOKUDEJO 187
	OLOWOKUDEJO 188
<i>B. scaposa</i> SENNEN ex MACH.-LAUR.	OLOWOKUDEJO 94, 95
	OLOWOKUDEJO 99
	OLOWOKUDEJO 100
<i>B. mediterranea</i> JORDAN	OLOWOKUDEJO 14
	OLOWOKUDEJO 15
<i>B. coronopifolia</i> L.	BG 780336
var. <i>coronopifolia</i>	BG 780429
var. <i>polyclada</i> (JORDAN) ined.	
<i>B. glacialis</i> (BOISS. & REUTER)	GÓMEZ-CAMPO
JORDAN var. <i>glacialis</i>	53-2161-72
	OLOWOKUDEJO 200
var. nov.	SOCORRO 209
<i>B. sempervirens</i> L.	OLOWOKUDEJO 120
	OLOWOKUDEJO 122
var. <i>brevifolia</i> (ROUY) HEYWOOD	OLOWOKUDEJO 232
<i>B. gredensis</i> GUINEA	GÓMEZ-CAMPO 2295
<i>B. sclerocarpa</i> REVEL	BG 180
<i>B. intermedia</i> GOUAN	GÓMEZ-CAMPO
	54-1199-68
<i>B. valentina</i> (L.) HEYWOOD	OLOWOKUDEJO 72
var. <i>valentina</i>	OLOWOKUDEJO 73
	OLOWOKUDEJO 82

reported in this paper. (For unstable chromosome numbers see Table 2.)

Locality	Chromosome number (2n)
Spain: Granada, Barranco de San Juan, valley of Río Genil, 1 200 m.	54 (53, 56)
Spain: Granada, Sierra Nevada/Guejar Sierra, 1 300 m.	54 (56, 57)
Spain: Granada, Sierrade Loja, 1 450 m.	54 (56, 57, 58)
Spain: Málaga, between El Burgo and Ronda, N 344 road near Ronda, 1 300 m.	54 (53, 57)
Spain: Málaga, Serranía de Ronda, 1 300 m.	54
Switzerland: Valais, Lac de Salanfè	36 (34)
Austria: Kärnten, Hohe Tauern	36
France: Dépt. Nord, Valenciennes	36 (34, 35)
Switzerland: Valais, 1 550 m	36 (37)
Switzerland: Saint Gall, Walensee	36 (37)
Switzerland: Alpes Valaisannes	36 (37)
Czechoslovakia: Montes Stracovska	18
Switzerland: Unterwald, Tribsee	18
Spain: Granada, Sierra de Loja, Cruz de Periqueta	18
Spain: Granada, Sierra de Loja, Santa Cruz de Alhama	18
Spain: Madrid, El Escorial, Valle de los Caidos, 1 100 m.	18
Spain: Madrid, Sierra de Malagón, 1 200 m.	18
Spain: Madrid, North of Las Herreras, 1 400 m.	18
Spain: Valencia, East of Játiva, 550 m.	18
Spain: Alicante Sierra de Carrascal, near Jijona	18
France: Vaucluse, Mont Ventoux	18
France: Drôme, Bourg-de-Péage	18
Spain: Granada, Sierra Nevada	18
Spain: Granada, Sierra Nevada, near Mulhacén, 3 480 m.	18
Spain: Granada, Sierra Harana, Cortijo del Sotillo	18
Spain: Granada, Sierra de la Yedra, N. of Alfacar, 1 200 m.	18
Spain: Granada, S. of Huerto Santillán, 1 100 m.	18
Spain: Almería, Sierra de Gador, near Dalias, 600 m.	18
Spain: Ávila, Circo de Gredos	18
France: Aveyron, Firmy, Puy de Wolf	18
Spain: Madrid, Sierra de Guadarrama	18
Spain: Jaén, Sierra del Pozo, Pico de Cabañas	18
Spain: Jaén, Sierra de Cazorra	18
Spain: Jaén, Sierra de la Cabrilla	18

Table 1 (continued)

Species	Collector/source and reference number
var. <i>leptophylla</i> (PAU.) ined.	OLOWOKUDEJO 6
	OLOWOKUDEJO 8
var. nov.	OLOWOKUDEJO 11
<i>B. auriculata</i> L.	OLOWOKUDEJO 7
	OLOWOKUDEJO 9
<i>B. cichoriifolia</i> LOISEL.	BG 780335
<i>B. didyma</i> L.	BG 7711425
	BG 7800337
	BG 7800339
<i>B. lyrata</i> L.	BG 771147
<i>B. baetica</i> BOISS. & REUTER	HEYWOOD & al. 74
<i>B. microcarpa</i> DC.	HEYWOOD & al. 138

Table 2. Variation in chromosome number of tetraploid *B. laevigata* s.l. (For provenances see Table 1.)

Locality	No. of plants counted	No. of individuals with chromosome no. of			
		34	35	36	37
Switzerland: BG 780349	13	1	0	12	0
Austria: BG 780416	4	0	0	4	0
France: BG 187	18	1	1	16	0
Switzerland: J. B. G. 780348	10	0	0	9	1
Switzerland: BG 780423	7	0	0	6	1
Switzerland: J. B. N. 780420	11	0	0	9	2
Total	63	2	1	56	4

an irregular (dysploid) series of $2n = 12, 16, 18, 36$ and 54 . The chromosomes of *Biscutella* are small and therefore detailed comparison of karyotypes has not been possible.

The two species of subgenus *Iondraba* (MEDIK.) COSSON both have $2n = 16$. This confirms the previous counts for *B. auriculata* L., while those for *B. cichoriifolia* LOISEL. are the first to be reported. These two species differ from other taxa of the genus by a series of distinct morphological characters and are sometimes treated as separate genus, *Iondraba* MEDIK.

Locality	Chromosome number (2n)
Spain: Cuenca, Madrid-Valencia road, near Alarcón	18
Spain: Cuenca, Motilla del Palancar	18
Spain: Valencia, N. W. of Requena, South of Utiel	18
Spain: Cuenca, Alarcón	16
Spain: Cuenca, Motilla del Palancar	16
France: Hautes Pyrenees, Bagnères de Luchon	16
Spain: Málaga, Ronda	16
Egypt: Alexandria	16
Greece: Attica, Athens	16
Italy: Sicilia, Siracusa	16
Spain: Cádiz, Castella de la Frontera-Almoraina	16
Spain: Cádiz, El Colorado, near Chiclana de la Frontera	12

Table 3. Variation in chromosome number of hexaploid *B. variegata* s. l.

Taxa	Origin of material (see Table 1)	No. of plants counted	No. of individuals with chromosome no. of				
			53	54	56	57	58
<i>B. variegata</i>							
var. <i>variegata</i>	Spain: OLOWOKUDEJO 60	17	1	14	2	0	0
	Spain: OLOWOKUDEJO 62	8	0	5	1	2	0
	Spain: OLOWOKUDEJO 88	9	0	6	1	1	1
<i>B. megacarpaea</i>	Spain: OLOWOKUDEJO 92	8	1	6	0	1	0
<i>B. foliosa</i>	Spain: OLOWOKUDEJO 93	5	0	5	0	0	0
	Total	47	2	36	4	4	1

The other subgenus, *Biscutella*, is divisible into two sections: sect. *Biscutella*, with five species, and sect. *Laevigatae*, which consists of 27 species, 12 subspecies and 21 varieties (LOWOKUDEJO 1980). Of the five species in section *Biscutella*, four have been examined cytologically in this study: *B. didyma* L., *B. lyrata* L., *B. baetica* BOISS. & REUTER and *B. microcarpa* DC. The first three have $2n = 16$, thus confirming previous counts. *B. microcarpa*, on the other hand, has $2n = 12$ —the lowest number encountered in the genus. This species is morphologically similar to *B. baetica* but is easily recognisable by its distinctly winged median filaments. The only perennial member of the section, *B. radicata*

COSSON, was not available for cytological examination but LARSEN & LAEGAARD (1971) recorded $n = 8$ for a collection from Sicily which they regarded as approaching this species. *B. radicata* has been reported from Sicily by HEYWOOD (1964) and by PIGNATTI (1982) and the plate in LARSEN & LAEGAARD's paper represents plants that may well be *B. radicata* so that this chromosome count is probably acceptable.

Section *Laevigatae* contains the largest number of taxa and, moreover, most of the taxonomic problems encountered in the genus are found here. Three sets of chromosome numbers, constituting a polyploid series of $2n = 18, 36$ and 54 , occur within this section.

Among the diploid taxa with $2n = 18$, the following are reported here for the first time: *B. sclerocarpa* REVEL, endemic to Aveyron, S. France; *B. intermedia* GOUAN, endemic to N. and C. Spain and the Pyrenees; a tall, large-fruited variety of *B. glacialis* (BOISS. & REUTER) JORDAN from the Sierra de Harana (Granada prov.); *B. sempervirens* L. var. *brevifolia* (ROUY) HEYWOOD from S. Spain; and *B. valentina* (L.) HEYWOOD var. *leptophylla* (PAU) ined. and a new pinnate-leaved variety, both from the Valencia region. Other diploid species for which counts are reported here are *B. gredensis* GUINEA, endemic to the Sierra de Gredos, C. Spain, confirming KÜPFER's (1968) earlier count of $2n = 18$, *B. frutescens* COSSON, a very distinct species endemic to Spain and the Balearic Islands, and *B. scaposa* SENNEN ex MACH.-LAUR., endemic to France (E. Pyrenees) and Spain, both confirming the counts of $n = 9$, $2n = 18$ by SCHÖNFELDER (1968); *B. mediterranea* JORDAN from S. France and E. Spain, and *B. coronopifolia* L. (incl. *B. polyclada* JORDAN), one of the most widespread and variable species, occurring in E. Spain, C. and S. France and N. W. Italy, confirming MANTON's (1937) counts of $2n = 18$ for the latter two species.

B. laevigata has been described as one of the most polymorphic species in the European flora (HEYWOOD 1964), containing up to twenty or more subspecies or varieties, and it is the only species in the genus to show intra-specific polyploidy. In this investigation counts have been made of ten populations, from Austria, Czechoslovakia, France and Switzerland, representing five subspecies. Three of these subspecies, *kernerii* MACH.-LAUR., *gracilis* MACH.-LAUR. and *guestphalica* MACH.-LAUR. (counts on garden material) were diploid with $2n = 18$, thus confirming previous reports. Tetraploid numbers were obtained for the remaining two subspecies *laevigata* and *lucida* (DC.) MACH.-LAUR., again agreeing with MANTON (1937) and with SCHÖNFELDER (1968). The possibility of misidentification is high in such a critical group, although other discrepancies have been reported in the literature. Thus DELAY (1971) published a report of $n = 9$ for *B. laevigata* subsp. *laevigata* [as subsp. *longifolia* (VILL.) ROUY & FOUC.] from Mont Cenis (Savoie,

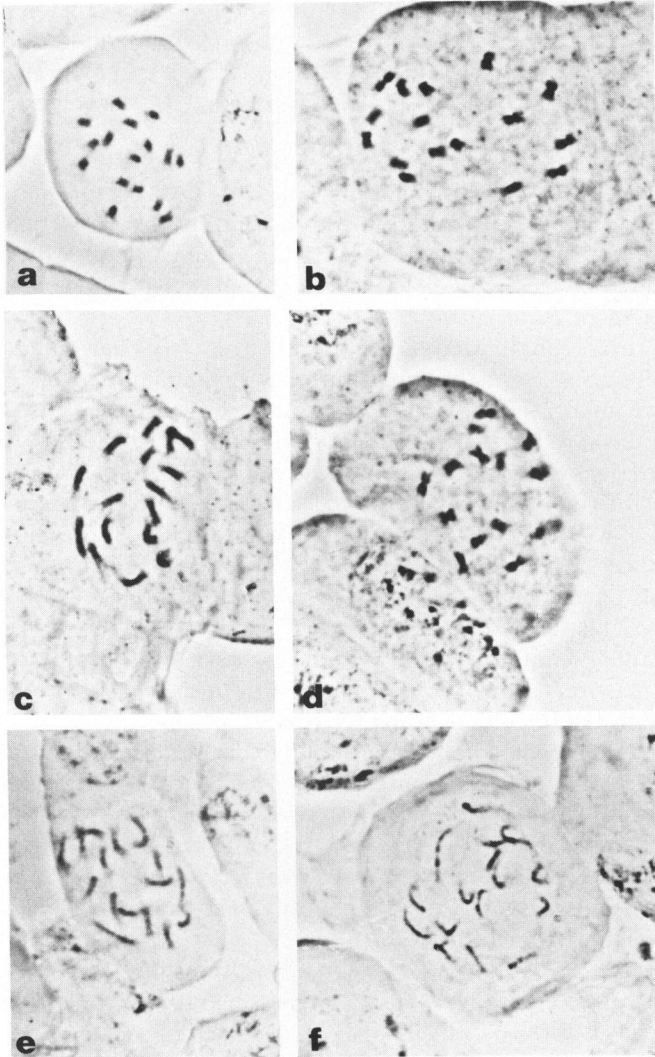


Fig. 1. Chromosomes of *Biscutella*. — a *B. baetica*, $2n = 16$ (HEYWOOD & al. 74); — b *B. lyrata*, $2n = 16$; — c *B. auriculata*, $2n = 16$ (OLWOKUDEJO 7); — d *B. didyma*, $2n = 16$ (two chromosomes barely visible at the lower edge); — e *B. cichoriifolia*, $2n = 16$; — f *B. cichoriifolia*, $2n = 16$. — All $\times 1300$

France) which disagrees with $n = 18$ or $2n = 36$ reported by previous authors, and HESS & al. (1970) give a count of $2n = 18$ for material reputedly of subsp. *tirolensis* (MACH.-LAUR.) HEYWOOD under the invalid combination "*B. tirolensis* (MACH.-LAUR.) stat. nov." from the Bergamasker Alpen (N. Italy) which conflicts with the earlier count of $2n = 36$ by MANTON (1934).

Ten per cent of the tetraploid plants investigated were found to contain either one or two chromosomes too few or too many, with $2n = 34, 35$ and 37 instead of 36 , as shown in Table 2. All the diploid populations examined were stable with a regular number of $2n = 18$. Similar cytological instability was found by MANTON (1934) in tetraploid populations of *B. laevigata*.

It is unfortunate that no material of *B. laevigata* from Spanish populations was available for cytological examination. On the other hand, an important feature of this investigation is the discovery of hexaploid counts in the *B. variegata* complex which, as we have already noted, is closely related to *B. laevigata* and largely replaces it in south Spain. All five populations of the *B. variegata* complex examined were hexaploid, with $2n = 54$. Apart from a count of $n = 8$ by SCHÖNFELDER (1968), these are the first reports for this southern Spanish species group and apply equally to the segregates *B. megacarpaea* BOISS. & REUTER and *B. foliosa* MACH.-LAUR.

Hexaploid counts have only been reported hitherto in three other species: MANTON (1937) recorded $2n = 54$ from specimens of *B. laevigata* collected in the Picos de Europa (N. Spain), while GARDE & MALHEIROS-GARDE (1953) reported the same number in material of *B. lusitanica* JORDAN from Portuguese material. Recently CASTROVIEJO (1982) has published a count of $2n = 54 + 0 - 2B$ in material of *B. vicentina* (SAMP.) ROTHM. ex GUINEA from Sagres, Algarve, Portugal. Although there is a fairly close relationship between *B. laevigata* and *B. variegata*, the other two hexaploid species are quite distinct from these and are not closely related to each other.

Interestingly 21 per cent of the plants examined of *B. variegata* were found to be cytologically unstable, possessing either $2n = 53, 56, 57$ or 58 , instead of $2n = 54$ (Table 3).

Breeding System. The flowers of *Biscutella* are unspecialized and are visited by a wide variety of insects. Nectary glands are well-developed in all the species examined. Under natural conditions the species flower from February to early October. The flowering period of the annual species, except *B. cichoriifolia*, is earlier (February to June) than that of most of the perennials (April to October). Field studies were made in the Sierra Loja, prov. Granada, S. Spain, of populations of *B. frutescens* and *B. variegata* var. *variegata* which were growing very close to each other.

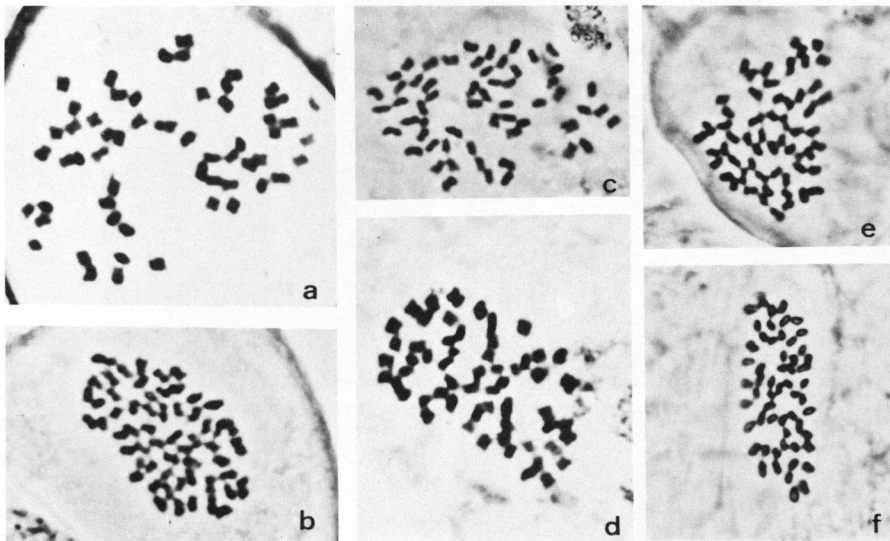


Fig. 2. Chromosomes of *Biscutella*. — a *B. variegata*, $2n = 54$ (OLOWOKUDEJO 60); — b *B. variegata*, $2n = 57$ (OLOWOKUDEJO 62); — c *B. variegata*, $2n = 56$ (OLOWOKUDEJO 88); — d *B. megacarpaea*, $2n = 53$ (OLOWOKUDEJO 92); — e *B. megacarpaea*, $2n = 57$ (OLOWOKUDEJO 92); — f *B. foliosa*, $2n = 54$ (OLOWOKUDEJO 93).—All $\times 1750$

They were effectively isolated by their different flowering times, *B. frutescens* flowering between April and late May and *B. variegata* var. *variegata* from June to August. No apparent hybrids were detected and no interspecific hybrids have so far been reported for *Biscutella* in the literature.

Self-compatibility tests were carried out and the results revealed that both self-compatible and self-incompatible species are present within the genus, as is found in most tribes of the *Cruciferae* (BATEMAN 1955 a). Most of the annuals such as *B. microcarpa*, *B. didyma*, *B. lyrata* and *B. auriculata* set plentiful seed on selfing. In *B. cichoriifolia* some plants set abundant fruits when selfed while others produced no fruits at all, suggesting that both systems occur in this species. The perennial species examined, such as *B. variegata* s. l., *B. scaposa*, *B. frutescens*, *B. glacialis*, *B. sempervirens*, *B. valentina*, *B. intermedia* and *B. laevigata* s. l. were found to be self-incompatible.

Four species of the genus, namely *B. variegata* var. *variegata*, *B. frutescens*, *B. coronopifolia* var. *coronopifolia* and *B. didyma*, were found to be strongly protogynous with their stigma often receptive for up to five days prior to anthesis. In *B. auriculata* and *B. cichoriifolia*, the

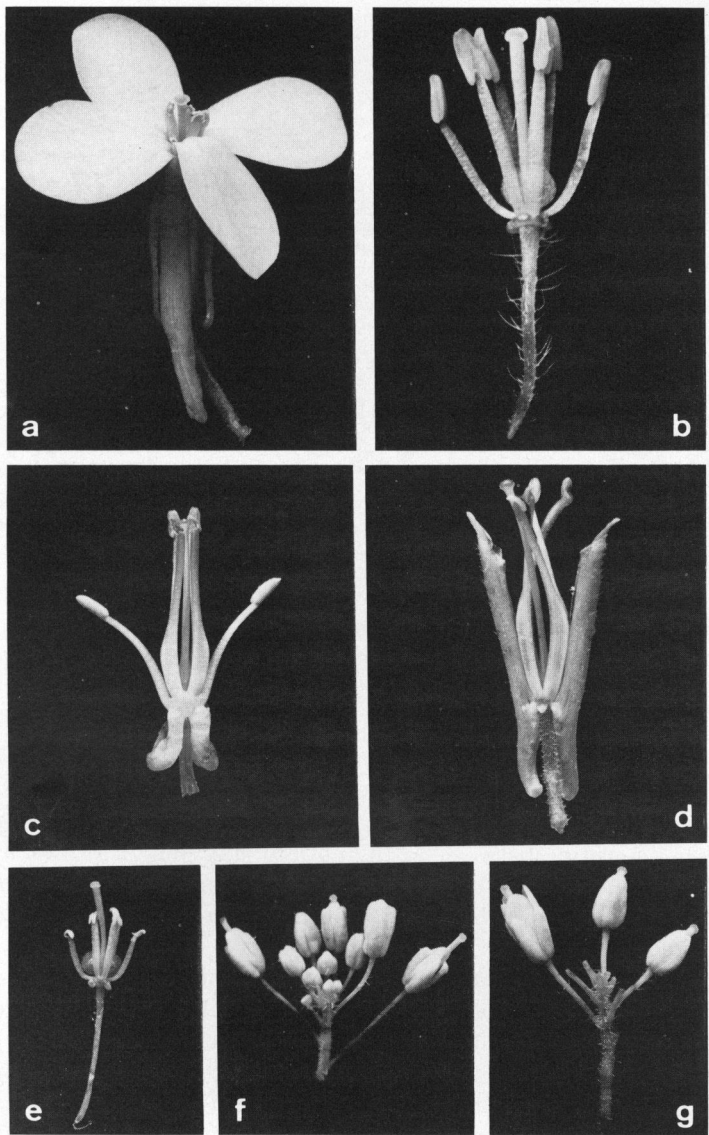


Fig. 3. Floral structure of *Biscutella* species. — *a* *B. cichoriifolia*: mature flower showing petals with patent limbs, a pouched sepal and the narrow median sepals ($\times 1.5$). — *b* *B. valentina*: mature flower with petals and sepals removed to show the tetradynamous nature of the stamens. Note the nectaries at the base and the extrorse position of median stamens ($\times 2$). — *c* *B. auriculata*: showing the pouched lateral sepals and the introrse position of the lateral stamens ($\times 2$). — *d* *B. cichoriifolia*: with petals removed to show the median nectary ($\times 2$). — *e* *B. valentina* var. *valentina*: petals and sepals removed to show the anther in a nearly horizontal position ($\times 1.5$). — *f* *B. frutescens*: with protogynous flowers ($\times 1.5$). — *g* *B. variegata*: with protogynous flowers ($\times 1.5$)

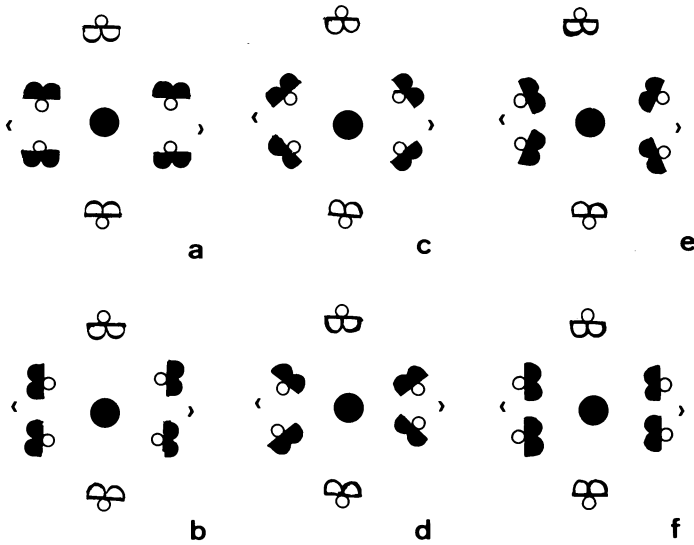


Fig. 4. Diagrams of stamen patterns with respect to the stigma in *Biscutella* species. The stigma is at the centre. The single lateral stamens are most remote from the stigma and their bilobed anthers are shown in white with the filament in each case shown as a white circle at the back. The pairs of median stamens are shown in black and indicated by a small bracket. —a The paired stamens are in an extrorse position with the anthers facing the direction of the short lateral stamens, e.g. *B. variegata* var. *variegata*, *B. valentina* and *B. lyrata*. —b The paired stamens are in an extrorse position but with the anthers fully turned away from the stigma and the lateral stamens, e.g. *B. variegata* var. *variegata*. —c The paired median stamens are still in an extrorse position but are rotated slightly so that they are at an angle to each other e.g. *B. coronopifolia* var. *coronopifolia*, *B. scaposa*, *B. glacialis*, *B. laevigata* subsp. *lucida*, *B. sempervirens* var. *brevifolia*, *B. frutescens*, *B. microcarpa*. —d The paired stamens are rotated slightly inwards until one of the anther sacs is nearly in an introrse position, e.g. *B. intermedia*. —e One of the anther sacs is in an introrse position, the other sac being partly orientated towards the lateral stamen, e.g. *B. auriculata*, *B. intermedia* and *B. lyrata*. —f The paired stamens are in a fully introrse position, e.g. *B. variegata* var. *variegata*, *B. didyma*, *B. auriculata*, *B. cichoriifolia*

structure of the flower ensures a very close association between the stigma and anthers (Fig. 3), which are held upright by the petal-claws and the spurred lateral sepals. However, the style may elongate in both species, thereby placing the stigma clearly above the anthers and reducing the contact between them.

A detailed study of the anther positions in relation to the stigma revealed that the two short lateral stamens always bear their anthers in the introrse position. In contrast the other two pairs of median stamens

bear their anthers in positions ranging from extrorse to introrse. The observed positions are represented diagrammatically in Fig. 4. The anther positions are fairly constant within a plant, but as the flower ages and the pollen chambers are almost empty, the anthers may often lie in a nearly horizontal position.

Discussion

Chromosome Numbers and Classification. The genus *Biscutella* is highly variable, both cytologically and morphologically. Polyploidy and aneuploidy have clearly played an important role in the evolution of the genus.

The basic chromosome number is taken to be $x = 9$ because it is the most likely number from which the polyploid series present in the genus could be derived. This basic number occurs in about 65 per cent of all the taxa which have been examined cytologically. Moreover, evidence from well-studied examples in other genera showed that reduction in number is much more frequent than increase, e.g. in *Crepis* (TOBGY 1943), *Hoplopappus* (JACKSON 1962) and *Chaenactis* (KHYOS 1965). It follows, therefore, that the diploid number of $2n = 16$ found in the two species of subgenus *Iondraba* (*B. auriculata* and *B. cichoriifolia*) could have arisen by the loss of one pair of chromosomes from the presumed original diploid number of $2n = 18$. They are easily separable from each other morphologically, ecologically and geographically. *B. auriculata* occurs in the Iberian peninsula, Balearic and Canary Islands and parts of North Africa where it grows in cultivated and abandoned farmlands. In contrast, *B. cichoriifolia* grows on rocky soils and dry sites which are unsuitable for cultivation in parts of Spain, France, Yugoslavia and Italy. These two species must have diverged very early during the evolution of the genus.

The diploid number, $2n = 16$, found in three species of subg. *Biscutella* sect. *Biscutella* series *Lyratae*, namely *B. didyma*, *B. lyrata* and *B. baetica*, could also have arisen by the loss of a pair of chromosomes associated with morphological changes which distinguish them from the species of series *Laevigatae*. The fourth species in this series, *B. microcarpa*, which has $2n = 12$ —the lowest recorded so far within the genus—must have originated by the loss of three pairs of chromosomes. Apart from this unique number, the distinctly winged filaments are sufficient to distinguish this species from all the others in the genus.

Section *Laevigatae* is taxonomically complex. The diploid ($2n = 18$) and tetraploid ($2n = 36$) populations comprising *B. laevigata* are poorly differentiated morphologically from each other. Microscopic examination of fruits, seed coats and pollen grains showed that the

diploids, as a group cannot be distinguished from the tetraploids by any one character or character-combination (OLWOKUDEJO 1980). MANTON (1937) suggested uniting all the diploids as one species and the tetraploids as another, a course of action supported by LAWALRÉE (1950) and LÖVE & LÖVE (1974), but rejected by HEYWOOD (1964) and by MARKGRAF (1963). This study has shown that there is such a continuous transition and overlap in the characters of the diploid and tetraploid assemblages that any attempted specific separation would be quite arbitrary. The taxonomic recognition of polyploid races showing slight morphological divergence is a subject which has provoked considerable controversy (HEYWOOD 1960, LÖVE 1960), although in recent years a less dogmatic approach has tended to be adopted. A compromise between biosystematic theory and taxonomic feasibility has to be sought.

Crossing experiments and analysis of hybrids have further shown that these polyploid populations are not separated by effective sterility barriers since the diploids can cross readily with the tetraploids to produce fertile triploid hybrids (MANTON 1937, OLWOKUDEJO 1980). Moreover, meiotic studies by MANTON (1937) of two tetraploids, subsp. *laevigata* and *lucida*, showed quadrivalent groups in sufficient number to suggest that the tetraploids are probably autopolyploids.

While polyploidy is no longer regarded as an automatic indicator of specific status, due recognition must be given to it as an important evolutionary mechanism and, by inference, of taxonomic significance. In this particular case, however, the differences in chromosome level do not correlate closely with overall morphological differentiation and it would serve no practical purpose to recognize the diploid populations as one species and the tetraploids as another since they cannot be distinguished morphologically from each other. This is not unexpected as it has been a common experience in other polyploid complexes following wide sampling. Furthermore, little is known of the cytological status of the Spanish and Portuguese populations of *B. laevigata* apart from the single hexaploid count by MANTON (1937) on material from the Picos de Europa discussed above. It was noted by HEYWOOD (1964) that these populations show considerable variation and that it would be possible to describe several regional variants in Spain and Portugal as distinct as those from other parts of the species' range.

In the light of the above considerations all the populations of *B. laevigata* are treated here as belonging to a single species containing a series of more or less differentiated subspecies, some of which are usually diploid, others usually tetraploid, following MARKGRAF (1963) and GUINEA & HEYWOOD (1964); further details are given in OLWOKUDEJO (1980).

An interesting feature of this investigation is the discovery that all

material of the *B. variegata* complex studied cytologically has proved to be hexaploid. This species is closely related to *B. laevigata*, differing mainly in its larger broader leaves and larger flowers and fruits. Many further counts are needed within the *B. variegata* complex to establish how consistent its hexaploid status is and to seek some insight into the origin of the number $2n = 54$. It could have arisen by crossing between diploid and tetraploid populations giving a triploid which was subsequently doubled. As we have seen, the only tetraploids recorded so far in the section *Laevigatae* are in *B. laevigata* which does occur in Spain, mainly in the north and centre. Although we do not know the cytological status of the Spanish populations it does seem likely that *B. laevigata* has been involved in the origin of *B. variegata*.

The presence of chromosomal imbalance in some tetraploid and hexaploid plants of *B. laevigata* and *B. variegata* does not appear to affect the success of these species as effective colonizers.

Although the aneuploid numbers in this genus are interpreted as reflecting a decreasing series, there is little experimental evidence to substantiate the direction of change in such cases, as ORNDUFF (1966) has pointed out. Conversely attention has been drawn to the reversibility of this phenomenon (RAVEN & THOMPSON 1964, DE WET 1968), but such cases are known to be very rare (JONES 1970). In general chromosomal changes, like those observed in *Biscutella*, are important, sometimes fundamental, steps in evolutionary change. However, the evidence obtained directly from studying chromosomes alone is seldom sufficient by itself to determine its direction.

Breeding System. The presence of self-compatible and self-incompatible taxa within the genus *Biscutella* has now been established. There is also the probable occurrence of both systems in *B. cichoriifolia*. This intraspecific phenomenon had previously been reported in the *Cruciferae* in two species of *Leavenworthia* by ROLLINS (1963 a).

A strong correlation was observed between annual habit and self-compatibility and also between the perennial habit and self-incompatibility. The latter phenomenon has been found by KIRCHNER (1905) in the *Papilionaceae*, by BEDDOWS (1931) in some grasses, and it has been discussed as a generalisation by STEBBINS (1958) and BAKER (1959). The self-incompatibility system which characterizes the perennial species may account for the largely continuous pattern of morphological variation found in sect. *Laevigatae*, since the gene-flow prevents, to some extent, the build-up of an integrated morphological pattern which is constant and clear enough to allow the easy recognition of species. The occurrence of both self-compatibility and self-incompatibility in most tribes of the *Cruciferae* has been pointed out by

BATEMAN (1955 a). The incompatibility system in this family has been shown to be sporophytically controlled (BATEMAN 1955 a, THOMPSON 1957, SAMPSON 1964, OCKENDON 1972), perhaps with multiple alleles at the S-locus.

The various anther positions described for *Biscutella* (Fig. 4) show no correlation with the breeding systems, unlike the situation in *Leavenworthia* where ROLLINS (1963 a) observed that the change from the extrorse to the introrse position was strongly correlated with the change from self-incompatibility to self-compatibility. The significance of the variations seen in *Biscutella* is not at all clear.

The presence of protogyny in four species of *Biscutella* is further evidence to counter the assertion by BATEMAN (1955 b) that "there is no protandry, no protogyny . . . in the *Cruciferae*". ROLLINS (1963 b) had already found that two species of *Streptanthus* were protandrous and it may well be that further research will reveal that such uneven maturation of the sex organs is more widespread than at present thought.

The breeding system in *Biscutella* is, therefore, quite complex. There is substantial inbreeding in the self-compatible annual species, with the positioning of the anthers and stigma in *B. auriculata* and *B. cichoriifolia* ensuring self-pollination. On the other hand, *B. didyma*, another self-compatible annual, has been shown to be protogynous; and the presence of well-developed nectary glands in all species of *Biscutella* suggests that some degree of outbreeding probably occurs in these annuals. The perennial species are mainly self-incompatible and, therefore, outbreeding; again protogyny is found in at least three of them.

Conclusions

The data presented in this paper have shown that the cytological pattern is more complex than previously realized. It has been confirmed that the species of subg. *Iondraba* are diploid with $2n = 16$ as are the species of subg. *Biscutella* sect. *Biscutella* with the exception of *B. microcarpa* with $2n = 12$. In sect. *Laevigatae* all species are diploid with $2n = 18$ with the exception of *B. laevigata* itself which comprises a series of mainly diploid ($2n = 18$) and mainly tetraploid ($2n = 36$) populations and *B. variegata* which was found to be uniformly hexaploid ($2n = 54$) in all samples examined. *B. variegata* is closely related to *B. laevigata* and it is suggested that it may have been derived from crosses between diploid and tetraploid races, involving the latter species, followed by chromosome doubling.

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