

**CYTOLOGY AND BREEDING BEHAVIOUR
OF GIANT ALIEN POLYGONUM SPECIES
IN BRITAIN.**

**A thesis submitted to the University
of Leicester for the degree
Doctor of Philosophy**

by

John Paul Bailey

B.Ed.

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CYTOLOGY AND BREEDING BEHAVIOUR OF GIANT ALIEN POLYGONUM

SPECIES IN BRITAIN.

J.P.BAILEY MAY 1989

An investigation into the cytology and breeding behaviour of the large asiatic Reynoutria species (R.japonica and R.sachalinensis), which were introduced into the British Isles in the last century and are now a significant component of the British Flora. A number of colonies have been examined morphologically and cytologically; hybrids have been identified and artificially re-synthesised in the laboratory. The sex-expression of the Reynoutria taxa has been examined, and has been found to be gynodioecious. R.japonica var. japonica has been found only as male-sterile plants in the British Isles, which has made it particularly susceptible to hybridisation with Fallopia baldschuanica (the commonly grown garden plant Russian Vine.) Seed production and viability, and the role of seed production in the colonization of Britain has also been investigated.

Chromosome counts and karyotypes have been produced of Reynoutria taxa and of the closely related Fallopia species. 2c DNA amounts have been determined, and Giemsa and fluorescent banding techniques employed.

A comprehensive synonymy has been produced, and the relationship between the genera Fallopia and Reynoutria discussed. One conclusion of this research is that the genus Reynoutria should be incorporated into the older genus Fallopia.

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Statement

The contents of this thesis are based on work conducted by the author in the Department of Botany, University of Leicester, mainly during the period October 1983 to October 1988.

The work recorded in this thesis is original, unless otherwise acknowledged in the text or the references.

signed...*J. P. Bailey*.....

DEDICATION

To my wife Angela, without whose assistance I would have been unable to undertake this project.

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Noms botaniques.	Clima.	Usage.	Prix suivant la force.		
			A	B	C
Lygodium japonicum Hort. Amst.	fr. b	Fougère.	2		
Lysimachia japonica Thbg.	c	Pl. pendante.	2		
Marsdenia tomentosa Morr. et Decais.	b	Pl. grimpe.	1		
Narcissus Tazetta Linn. var. jap.	c			5	
Nipholobus Lingua Spreng.	b	Fougère.	2		
Onchium japonicum Hort.	b	id.			
Ophiopogon Jaburan Sieb.	c	Pl. d'ornem.		2	
— japonicus Kerr.	c	Pl. d'orn. méd.		1	
— spicatus Kerr.	c	Pl. d'ornem.		2	
Opuntia Saboten Sieb.	b	id.		5	
*Ornithocrotalum japonicum S. et de Vries.	c	id.	2	5	
— — foliis aurovariegat.	c				
*Phajus maculatus Lindl.	b	Orchidée.			
— minor Bl.	b	id.			
Polygonatum japonicum Morr. et Decais. 1)	c	Pl. d'orn. et us.		1	
— Thunbergii Morr. et Decais.					
— — *foliis margin.					
*Paeonia albiflora Pallas. fl. alb.	c	Pl. d'ornem.		1	
— — la douzaine de différentes variétés		id.		6	
Pardanthus chinensis Kerr. var. jap.	c	id.		1	
Polygonum filiforme Thbg.	c	id.		1	
— pictum Sieb.	c	id.		1	
— Sieboldii Reinw. 2)	c	Pl. d'or. et four.			1
— — la douzaine					3
— — le cent.					25
Rhodes japonica Roth.	b	Pl. d'ornem.		2	
— — variegata	b	id.		3	
— — marginata	b	id.	5		
— — minor	b	id.	2		
— — *macrophylla	b	id.			
Roxburghia phyllantha S. et Z. var. officinalis	b	Pl. bot. et méd.	5		
Rupifraga sarmentosa Thbg.	c	Pl. d'or. et pen.			1
— minor	c				1
Scutellaria japonica Morr. et Decais.	c	Pl. d'ornem.		1	
Sedum Sieboldii Sweet.	c	Pl. pendante.		1	
Splitgerberia japonica Micq.	b	Pl. d'ornem.		1	
Thermopsis fabacea D.C. var. jap.	c	id.		1	
Tradescantia discolor Smth. var. elatior	a	id.		10	
Urtica nivea Linn.	b	Pl. usuelle.		2	
Viola Patrinii D.C.	c	Pl. botanique.		2	
Vincetoxicum atratum Bge.	c	Pl. d'ornem.		2	
— japonicum Morr. et Decais. & flavescens	c	Pl. grimpante.		1	

1) Les racines sont mangées comme chez nous les Asperges.

2) Cette Renouée est une de nos introductions les plus importantes du Japon, une plante d'ornement vivace, inextirpable, d'un feuillage luisant et des fleurs en grappe très gracieuses, par laquelle on peut improviser des bosquets, abriter les jeunes plantations et fortifier les collines sablonneuses et les dunes. L'herbe qu'on peut faucher en printemps à plusieurs reprises fournit un fourrage excellent pour l'engraissement des bestiaux qui la mangent de préférence; les fleurs, qui paraissent en automne, sont très mielleuses et donnent aux abeilles leur provision d'hiver; la racine amère et tonique est un médicament réputé chez les Chinois et les Japonais; enfin les tiges même qui meurent en hiver sont bonnes à brûler et pour en faire des allumettes.

FRONTISPIECE

Page from the 1856 catalogue of von Siebold and Company Leiden, featuring Polygonum Sieboldii Reinw. (= R. japonica) and a footnote (2) extolling the plant's considerable virtues!

Chapter 1

Introduction

1.1 STARTING POINT

This study has its origins in a problem encountered by Ann Conolly in her work on the distribution of the alien rhizomatous perennials Reynoutria japonica Houtt. (= Polygonum cuspidatum Sieb. et Zucc.) and Reynoutria sachalinensis (F. Schm. ex Maxim) Nakai (= Polygonum sachalinense F. Schm. ex Maxim.) in the British Isles (Conolly, 1977). She found that, whilst most specimens could be readily attributed to one or other taxon, certain ones appeared to be intermediate. An examination of the ploidy level of these plants revealed in most cases a chromosome number that was also intermediate and strongly suggestive of hybrid origin (Bailey and Conolly, 1985). Other intriguing points were also raised by Conolly (1977). These related to the sex expression and the part played by seed production in the invasion of the British Isles by these two species. Commonly described as functionally dioecious there exists in R. japonica in Britain a super abundance of male-sterile plants. This is not the case in R. sachalinensis, where male-fertile plants are more commonly found, some of which are capable of setting seed. At the time of publication of Conolly's work there were few records of seed-set in these species. Reference was made to the beneficial effect of long hot summers, or the detrimental effect of an early frost, but at that time it was not known whether or not such seed as was produced was fertile, and there were certainly no records of seedlings becoming established in the wild. Conolly concluded with the remark that 'Gaps and problems remain...' and expressed

the hope that her work would act 'as an incentive and stimulus to the assembling of further data and, in particular, further investigation into the behaviour and incidence of the sexual variants in R. sachalinensis..'. It is to these gaps and problems that a major part of this study is addressed.

1.2 THE POLYGONACEAE

The Polygonaceae are a family of some 800-1000 species spread between about 20 and 40 genera. The family has a cosmopolitan distribution, not only geographically but also ecologically. Its members may be found in arctic, temperate and tropical climes, in deserts, swamps and on mountain tops. There is a similar variation in growth-form. Whilst predominantly herbaceous (and mostly perennial), there are also some shrubs, trees and woody and herbaceous climbers, as well as many annuals. The family does not contain many economically important species, the best known probably being buckwheat (Fagopyrum esculentum Moench.). This is, according to Simmonds (1976), a crop of secondary importance, and although cultivation is decreasing it is still grown in N. E. India, China, USSR, North America and France. However, the current vogue for health foods has seen some increase recently in the acreage grown in Canada after years of decline. Though not of course a cereal, it is used in a similar way, the dehulled achenes, being ground to produce a flour which is then used to make pancakes and noodles etc. Fallopia multiflora (Thunb.) Haraldson and to a lesser extent R. japonica are valued in the East for their tonic rhizomes. The petioles of rhubarb (Rheum cultorum Thorsrud and Reis.) are eaten widely, as are the leaves of various Rumex and Polygonum species as pot herbs or in salads. A number of species find their way into gardens, e.g. Fallopia baldschuanica (Regel) Holub, Polygonum campanulatum Hook. and P. bistorta L. as ornamentals; F. convolvulus (L.) Love, R. sachalinensis and R. japonica as

invasive and persistent weeds.

Whilst most recent classifications are in agreement that the Polygonaceae are an isolated and well defined family that is the sole member of the order Polygonales (Cronquist (1981), Dahlgren (1977), Takhtajan (1980) and Thorne (1983)), there is less agreement over its precise position in the hierarchy. Cronquist (1981) thought their affinities lie with the Caryophyllales and accordingly placed the Polygonales between Caryophyllales and the Plumbaginales; a position more or less in agreement with that of Thorne and Takhtajan. Dahlgren (1977), however, favoured the amalgamation of Plumbaginales and the Polygonales at a higher level, and placed the Plumbaginae between the Balanophoranae and the Primulanae, as he considered the Caryophyllales to be clearly distinct from the Polygonales and the Plumbaginales.

The taxa that are the concern of this study are found in the tribe Polygoneae of the sub family Polygonoideae. Traditional classifications of the family (Table 6.1) maintain all 200 or more taxa within the genus Polygonum, with sub-divisions at the sectional level (Table 1.1).

Increasingly though it has been considered that the group is rather unwieldy with 200 or so species, and is not in any case a very homogenous grouping. Accordingly the modern trend has been to take out some of the more obviously distinct segregates and to give them generic rank. The size and composition of the remainder has been subjected to various treatments. The taxa that are the primary concern

Polygonum L.

- sect. Polygonum (type sp.: P. aviculare L.)
 sect. Persicaria (type sp.: P. persicaria L.)
 sect. Bistorta (type sp.: P. bistorta L.)
 sect. Aconogonon (type sp.: P. divaricatum)

Fallopia Adans. (Bilderdykia Dumort.)

- | | |
|---------------------------|-----------------------------|
| sect. <u>Fallopia</u> | <u>F. scandens</u> (type) |
| | <u>F. convolvulus</u> |
| | <u>F. dumetorum</u> |
| sect. <u>Paragonon</u> | <u>F. cilinodis</u> (type) |
| | <u>F. cynanchoides</u> |
| sect. <u>Pleuropterus</u> | <u>F. multiflora</u> (type) |
| | <u>F. baldschuanica</u> |

Reynoutria Houtt.

- R. japonica (type)
R. sachalinensis

TABLE 1.1 CLASSIFICATION OF MAIN TAXA
 REFERRED TO IN THIS STUDY

of this work, whilst often united in the sectional treatment under section Tiniaria Meissn. are usually split into two genera Reynoutria and Fallopia. For the moment I shall use the taxonomic treatment of Flora Europaea (Tutin 1964) where section Tiniaria is treated as two genera separated from Polygonum: Reynoutria and Bilderdykia Dumort. (Table 1.1). Although the distinctions between these two are not very pronounced, I believe that they are both readily separable from the rest of Polygonum. In using the generic name Fallopia I am accepting Holub's (1971) argument that it is the correct name for the genus named in Flora Europaea as Bilderdykia.

1.2.1 REYNOUTRIA

The genus Reynoutria Houtt. (1777) is an old one and was described by Houttuyn from material sent back from Japan by Thunberg. Initially monotypic, it acquired a second species, R. sachalinensis, from the Sakhalin Islands in 1859. Since then various varieties have been described, most of them, incidently, named after regions of Japan and China. The most commonly encountered is R. japonica var. compacta (Hook. f.) Moldenke, a dwarf mountain variant that is still sold as a garden plant in this country. These taxa were originally introduced as garden plants in the last century, probably originating from the nursery of P.F. von Siebold in Leiden. The description of R. japonica given in his 1856 catalogue puts the claims of contemporary nursery men into the shade (see Frontispiece). He stated that this was one of the most important introductions from Japan. A

vigorous ornamental, with luxurious edible foliage for cattle, and attractive to bees which will swarm to its melliferous flowers in Autumn. It could be used to stabilize sand dunes and shelter plantations of young trees. Even the rhizomes were reputed to have tonic properties, and the attractive dry red stems could be used to make matches! Incidentally he omitted to say that the young shoots could also be cooked and eaten (Locandro, 1978), and that they have a 'very unique distinct almond-like flavour'. It is interesting to note that Locandro also mentioned that it is used in the U.S.A. to stabilize dunes, and it may be that some of the British coastal colonies originate from such a notion.

The Reynoutrias are large, rhizomatous herbaceous perennials; in suitable conditions R. sachalinensis may exceed 3 metres in height with leaves 40cm long. They flower in the Autumn with a mass of small flowers, which if fertilized produce a small trigonous achene enclosed in a persistent winged fruiting perianth. Although the rhizomatous habit and large size are found in some Himalayan species of sect. Aconogonon, the winged flowers and trifid fimbriate stigmas are otherwise limited to the genus Fallopia. These plants are now a widespread, if little noticed feature of the British Flora; their fall from favour as garden plants is not difficult to comprehend. One can well imagine the pleasure and delight of the Victorian gardener that such an exotic vigorous and attractive plant could be grown with such consummate ease in the British climate. Equally one can appreciate how such feelings could

turn to despair when any attempt to restrict this fine new plant would lead to the discovery of metres of inextirpable rhizomes, and a flush of new growth in unexpected places. It is small wonder then that such a plant should rapidly find itself dumped on waste ground. Conolly (1977) gives the first record for an 'escape' in the 1890s, where the colonisation process would begin. Conolly charted the rapid spread of this plant through the British Isles, and today there can be few 10km grid squares without it. R. sachalinensis, although a larger plant and equally rhizomatous, is not as invasive as R. japonica, and where it is found the stands tend to cover smaller areas.

The threat posed to the natural environment by R. japonica is at its greatest in parts of Wales, where as Conolly suggests the climate may be particularly suitable for a species originating from an oceanic area. River banks in west Wales are a particularly characteristic habit, Taylor (1987) reported that many miles of the river Teifi in Glamorgan are dominated by this species, whilst in Cardiff a postgraduate student has received some funding from the Welsh Water Authority to examine ways of preventing R. japonica rhizomes from forcing up the concrete blocks placed along rivers by the Authority as part of their flood prevention schemes. Unfortunately the predeliction for river-side sites places severe restrictions on the herbicides that may be used in such situations. Several studies^{by} Scott and Marrs (1984), Poore (1982) and Jackson and Turtle (1986) have examined methods of chemical or physical control of Japanese knotweed, and whilst repeated

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applications of Picloram, Broadshot and glyphosate will eventually eradicate colonies, it is doubtful if these can be used alongside water-courses. Jackson and Turtle (1986) reported that in the Snowdonia National Park regular cutting twice a year is the only cost-effective means of control. Even the disposal of cut stems may pose problems since Conolly (1977) reported regrowth from cut stems floating in rivers! It is not only the countryside that is at risk; grave yards, parks and car-parks may also be overrun. It must be some measure of the concern generated by R. japonica that it is singled out (along with one other land plant) by the Wildlife and Countryside Act (1981), which makes it an offence to introduce Japanese knotweed into the wild.

However, all this vegetative vigour is not without a positive side, for R. japonica, along with various other native or introduced plants, has been investigated as a possible source of biomass production. The Institute of Terrestrial Ecology at Merlewood have produced a number of reports on the feasibility of harvesting 'indigenous' plants and using the resulting biomass for conversion into energy by various means. Yields of up to 25 dry t ha⁻¹ reported by Callaghan et al. (1981) from R. japonica growing on waste ground in Manchester encouraged further research; not surprisingly, since this figure exceeded the biomass production of Italian Rye grass at Aberystwyth. However, the results from experimental planting of the two Reynoutria species reported by Callaghan et al. (1984) failed to live up to those high expectations.

Surprisingly, difficulties were encountered in getting the plants established for, despite a very high survival rate amongst the unshooted rhizomes used, competition from the local weeds proved to be a problem and much lower yields resulted. There is a possibility that the climatic conditions at the transplant site were not ideal, and also that the use of more vigorous clones may have given better establishment and higher yields. Indeed the authors suggested that a depauperate gene pool is found in Reynoutria species in Britain due to the absence of sexual reproduction, and that ample scope exists for the breeding and selection of more productive genotypes. They concluded that the 'Almost total lack of data on these wild species, one of which occurs only infrequently in the UK, led to many problems...' From these remarks it may be seen that research into the breeding behaviour, population structure, incidence of seed-set, and identification of potentially vigorous hybrid taxa would be of considerable service to any further assays of the biomass potential of the Reynoutrias.

All the Reynoutria taxa so far examined are polyploid at the tetraploid or octoploid level: R. japonica ($2n=44, 88$) and R. sachalinensis ($2n=44$). It is rather unusual to find a genus with only tetraploids and octoploids, however, the closely related Fallopia (which is often united with Reynoutria in section Tiniaria) contains mainly diploid taxa.

1.2.2 FALLOPIA

In contrast to the strictly erect herbaceous perennial nature of the Reynoutria taxa, Fallopia exhibits rather more diversity of life form. The unifying feature is the climbing habit, though annual and woody and herbaceous perennial species are found. This relatively greater diversity than Reynoutria is mirrored by the much wider geographical distribution of Fallopia taxa. Whilst of predominantly East Asiatic distribution, where most of the taxa are found, the annual members enjoy a much wider distribution. F. convolvulus has a Northern Circumpolar distribution, though it occurs only as a weed throughout its range. F. scandens (L.) Holub is found in North America and throughout the whole of Asia, whilst F. dumetorum (L.) Holub is found in Europe, North America and Asia.

Morphologically, Fallopia shares with Reynoutria the winged or keeled outer perianth segments a feature unique within Polygonum s.l. to these two genera. Whilst some Fallopia taxa have the trifid fimbriate stigma of Reynoutria most are characterised by a trifid capitate stigma. What is more the Fallopia taxa apparently all have hermaphrodite flowers and most are self-compatible in contrast to the separation of the sexes in Reynoutria.

In Britain the native species are the common agricultural and garden weed F. convolvulus and the much more restricted F. dumetorum. Sometimes listed as introduced taxa are the commonly grown 'Russian vines' or 'mile a minute vine', F.

baldschuanica (Regel) Holub and F. auberti (Henry) Holub. These plants have the vigour (but not the rhizomes) of Reynoutria taxa, and are often advertised on this basis for covering unsightly garden buildings such as neighbours' garages. Viable seed is rarely produced and I suspect that some of the 'naturalised' plants have simply grown out of an adjacent garden and subsequently rooted. F. baldschuanica reached Europe as seed in the last decade of the 19th century, F. aubertii following a decade later. Being easily propagated from cuttings there is probably a very limited genetic base in the plants in this country. The two taxa are extremely similar and as will be discussed later I am treating them as a single species F. baldschuanica.

The rhizomatous perennial F. multiflora is sometimes grown as a garden plant, mostly for its attractive foliage, for despite its name it is difficult to obtain flowers.

My interest in this genus has centred on the perennial taxa, since they are obviously more closely related to Reynoutria, and further because I have been unable to obtain living material of F. cynanchoides (Hemsley) Holub. and F. scandens -an important consideration in a cytotaxonomic study.

1.3 AIMS

The overall aim of this research is an examination of the inter and intra specific relationships of Reynoutria taxa and the relationship between Reynoutria and Fallopia. Evidence will be taken from cytology, morphology and from artificial hybridizations. At the same time it is a study of some of the consequences of the introduction of a small group of Asian perennials. Indications suggesting that these two genera might be more closely linked than hitherto believed has led to work on the natural distribution of these taxa, an examination of type material and the compilation of a comprehensive synonymy.

I have explained my initial involvement with Reynoutria and, since my main interests are cytological, I have attempted to bring cytological techniques to bear on this taxonomic problem. Unfortunately these taxa, particularly the Reynoutria, with their small rather numerous, more or less metacentric chromosomes and the high tannin content of their tissues, are difficult subjects for most types of cytological analysis. The first object has been to establish the range of chromosome numbers present in the British material, no previous data in this field being available. A large number of sites throughout the British Isles has been examined, and I have been fortunate enough to have the large collection of Reynoutria plants made over the years by Ann Conolly. Carrying on from this I have obtained material from Japan and China and examined the cytological literature. Karyotype analysis, meiotic analysis,

microdensitometry and various banding techniques have also been attempted.

The examination of breeding behaviour has been concerned with an investigation of the sex structure of the British populations; and also of the sex expression, pollen fertility and seed production of these plants. The incidence of naturally occurring hybrids and the production of artificial hybrids for comparison has also been carried out as has cytological investigation of the various hybrids.

The work is presented as self-contained chapters based on particular subject areas, and hence each contains the relevant introduction, materials and methods, results and discussion.

Chapter 2

Morphology and distribution

2.1 INTRODUCTION

Since this study is primarily concerned with cytology and to a lesser extent with breeding behaviour, anatomical and morphological aspects have been kept to a minimum and this chapter is therefore rather brief. Type descriptions and detailed morphological descriptions of the various taxa are presented along with ecological details where available. Anatomical investigation has been limited to the trichomes and cuticular patterning. An introduction to flower structure is given here, but detailed results of the shape and size of the flower components will be given in the Breeding Behaviour section.

Detailed distribution maps prepared from an examination of herbarium specimens from Kew, the British Museum and Edinburgh are given for those taxa whose distribution is primarily Asiatic. Many problems are encountered when trying to decipher the often illegible collection details on old herbarium sheets (particularly when they may be in any of three different languages and have been the subject of any one of a number of transliteration systems). This has meant that relatively few of the specimens examined have actually given rise to a location on a base map.

2.2 SPECIES DESCRIPTIONS

Reynoutria japonica Houtt. Handl. Regn. Veg. viii 639 t.51
f.1 (1777)

Type Description

R E Y N O U T R I A.

Naar zekeren Heer van REYNOUTRE, waar van LOBEL getuigt, dat dezelve aan de Kruidkunde zeer veel dienst gedaan heeft; zo als ik bevoorrens heb gemeld (*).

IV. Immers in dit Kruid heb ik de Bloempjes be-
AFDEEL. XI. vonden tien Meeldraadjes of liever Meelknopjes
HOOFD- te bevatten, en het Vrugtbeginzel is driekantig,
STUK. met drie Stempels gekroond: uit welken hoofde,
Driewy- anders, dit Gewas tot het Duizendknoop be-
rige. trokken zou kunnen worden. Het heeft den
Kelk vyfbladig, zonder Bloembladjes.

I. De eenigste Soort, my daar van bekend, heeft
Reynoutria een vooze, ronde, doch eenigszins gegroefde
Japonica. of gestreepte, bruinachtige Steng, die bogtig
PL. LI. is en knoopig, overhoeks Takken uitgeevende,
Fig. 1. welke, zo wel als de Steng, overhoeks bezet
zyn met lang gesteelde Piekswys' Hartvormige
Bladen, de grootsten wel vier Duimen lang en
derdhalf Duim breed. Hier en daar komen aan
de Knoopen, en in de Oxels der Bladen, drie
of vier Ristachtige Bloem- en Zaadtrosjes voort,
met zeer kleine Bloempjes, van gezegde hoe-
danigheid. De Bladen verkleinen niet naar bo-
ven, maar komen zelfs naar 't end der Takken

Translation

This is named after a certain Mr. Reynoutre who according to
Lobel worked with herbs, and whom I have already referred to.
I have found that these plants contain ten anthers shaped

like little buttons, and a trigonous gynoecium with 3 stigmata. If it was not for that it might be classified as a knotweed. The flower has five 'tepals' (leaves without flowering bits), the only species which I know has a hollow brown stem which is brown and ribbed. The stem has little bumps and is flexuous (zig-zags) the branches start from these nodes and are just like the stem and contain leaves at the nodes. Leaves heartshaped 'spießsweis' of which the largest are 3.5-4 'zoll', sometimes at these nodes or in the leaf axils are borne 3 or 4 thinner flowering branches with tiny flowers as mentioned above. Leaves do not get smaller towards the top, in fact they appear larger towards the end of the branch.

Herbaceous perennial with long woody rhizomes; stems erect, stout, hollow, up to 3m, 2-3cm diameter; leaves glabrous petiolate, ovate to orbicular abruptly acuminate at the tip, truncate at base (some Chinese material cuneate at base) 5-12x5-8cm. Inflorescence in axillary and terminal panicles; flowers borne in ochreate clusters of 3-6, small, white; outer 3 tepals winged; stamens 8, fat and well exerted in male-fertile plants, small, flat empty and included within perianth in male-sterile plants; stigma trifid, fimbriate (Plate 2.7a) protruding from the perianth in male-sterile plants, in male-fertile plants gynoecium minute surmounted by trifid immature stigma; fruiting perianth greatly enlarged and conspicuously winged, completely enclosing the brown shining trigonous achene.

This taxon occupies a number of habitats in Japan (I have no

ecological information on Chinese plants): Sunny places on hills and mountains (Ohwi, 1965), in shrub and herb layers of Salix sachalinensis forests (Kinosita 1987), and as one of the first colonizers of volcanic ash, growing within 300m of active craters (Numata 1974).

Distribution: China, Japan, Korea and Taiwan (see Figures 2.3 and 2.8), now widely naturalized in Britain, Europe and the U.S.A.

Varieties

A number of varieties are described in the literature (see nomenclature chapter for details) of which I am currently only recognising one, R. japonica var. compacta (Hook.f.) Moldenke, (Bull. Torr. bot. Cl. 68: p675, 1941). This taxon is very similar to R. japonica var. japonica but is distinguishable on the basis of its smaller stature usually less than 70cm, its smaller broader, leathery leaves with dark red petioles and crimped margins. The existence of plants of this description but taller suggests that this variety might actually grade into R. japonica proper, but confirmation of this would require an intensive examination of live Japanese material.

Reynoutria sachalinensis (F. Schm. ex Maxim) Nakai

Type description Schmidt ex Maxim. in Mem. Acad. Sci. St. Petersburg., 9:233 (1859)

(634) 20. **Polygonum (Tiniaria) sachalinense F. Schmidt.** Perenne (?), caule erecto valido glabro angulosostriato simplici (?); foliis breviter petiolatis late ovatis vel ovato-oblongis acuminatis: inferioribus basi subcordatis, superioribus truncatis, omnibus discoloribus subtus elevato-reticulatis; ochreis elongatis glabris membranaceis nervosis demum fissis; racemis axillaribus singulis vel fasciculatis folio multo brevioribus compositis densifloris; bracteis ovatis longe acuminatis paucifloris; rhachi crassiuscula ramulisque fuscotomentosulis; pedicellis capillaribus infra medium articulatis perigonio fructifero trialato brevioribus; alis caryopsi triquetra elliptica acuta nitida spadicea latioribus; stylis tribus brevissimis discretis inclusis, stigmatibus brevissime fimbriatis.

Herbaceous perennial with long woody rhizomes; very similar to R. japonica but larger; stems erect, stout, hollow up to 4m, to 3cm diameter; leaves ovate to oblong, larger ones cordate at base (upper inflorescence leaves may be truncate), 10-40 x 25cm, the larger leaves with long flexuous uniseriate hairs on undersides. Inflorescence in axillary and terminal panicles, denser than in R. japonica; flowers as in R. japonica but yellowy-green, stigmata usually more fimbriate.

Ohwi (1965) gave the occurrence of this species as along ravines and streams in mountains. Steward (1930) on the other hand suggested a coastal distribution. Numata (1974) reported it in seaside scree vegetation and as a colonizer of the unstable soil at the base of volcanic domes, and as a component of the shrub layer in pioneer forests on the lower slopes of volcanoes.

Distribution: Sakhalin Island (USSR), Hokkaido, the Japan Sea side of Northern and Central Honshu (Japan), the South Kurile Islands (USSR: chain of islands between Japan and Kamchatka) and Ullungdo Island (Korea). Also now widely naturalized in the British Isles mainland and the U.S.A.

Fallopia baldschuanica

Type description: Regel, Acta. Hort. Petrop., 8:684,
pl.10(1884)

81. *Polygonum baldschuanicum* Rgl.

Tabula X.

Glabrum; caule frutescente, lignoso, volubili, basi usque 13 Mm. in diametro, cortico fusco lenticellis crebris ornato. Ochreae brevissime cylindricae, ut bractae membranaceo-hyalinae mox fissae, denique subevanescentes. Folia inferiora opposita, longe petiolata, hastato-cordata, acuta v. acuminata v. rarius obtusa, margine obsolete crenulato-scabra, petiolum superantia; folia suprema floraliaque valde minora, nunc caulinis similia, nunc lineari-lanceolata in petiolum attenuata. Florum paniculae axillares terminalesque, folium pluries superantes, fere aphyllae, laxae; rhachis angulata, ad angulos minute scabra. Flores ramulorum paniculae fasciculati, pedicellati; fasciculi pluriflori; pedicelli supra basin articulati, apicem versus hyalino-albido trialati. Calycis 5-partiti hyalino-albidi lobis tribus exterioribus ovatis, patentibus, dorso naviculari-alatis; alis in pedicellum decurrentibus; sepalis interioribus duobus erectis, obovatis, exalatis, rubescentibus. Stamina 8. Stigma capitatum, trilobum,

in ovarii apice sessile. Calyx fructifer vix auctus, late alatus. Achaenium triquetrum, nigrum, nitidum.

Folia caulina incluso petiolo usque 7—8 Cm. longa; lamina usque 4 Cm. lata et 5 Cm. longa. Flores 5—6 Mm. in diametro. Caulis 10—15 pedes altus.

In Bucharae orientalis chanato Baldschuan ad fluvium Wachs ad pedem orientalem montium Sevistan, 4—5000' alt., mense Julio anno 1883, leg. A. Regel.

Species proxima *P. multiflorum* Thbrg. «caule herbaceo, floribus triplo minoribus rufescentibus, calyce fructifero valde aucto fuscescente alato» facile dignoscitur.

Woody perennial without rhizomes; stems climbing and trailing, extending to many metres with numerous lenticels on older regions; leaves glabrous, petiolate, blades

oblong-ovate, cordate at base, margin crenulate with one or two sinuses 2.5-9 x 2-5cm usually borne in clusters; inflorescence of branching panicles terminating the branches; flowers borne in ochreate clusters, quite conspicuous, white with green veins, 3-5mm diameter when first open; outer 3 tepals winged, stamens 8, base of filaments pubescent, stigma 3-lobed capitate; fruiting perianth greatly enlarged and conspicuously winged, to 12x10mm completely enclosing dull black trigonous achene with concave faces.

Habit; Thickets, wood margins, boulders and cliffs up to 2,500m.

Distribution: Western China, Tibet Western Pakistan and Afghanistan (Figures 2.2,2.4), also widely grown as a garden plant in Europe.

Fallopia multiflora (Thunb.) Haraldson

Type description: Thunberg, Fl. jap.:169 (1784)

*multi- P. foliis cordatis, caule volubili angulato, florum pani-
florum. cula ramosa.*

Japonice: Asju.

Crescit iuxta Nagasaki.

Flores Augusto, Septembri.

Caulis inferne scandens, angulatus, hinc inde flexus, laevis; superne volubilis, filiformis, glaber.

Folia alterna, petiolata, subcordata, ovata, acuminata, integra, glabra; inferiora sesquipollicem lata, basi subtruncata, bipollicaria; superiora magis cordata, minora.

Flores ex axillis foliorum paniculato-racemosi.

Panicula composita, patentissima.

Pedunculi et pedicelli capillares, divaricati.

Braetene ovatae, acutae, integrae.

Differt a P. dumetorum, cui valde similis:

1. floribus minutis, obtusis.

2. panicula ramosa, racemosa, patentissima.

Radix tuberosa, subcarnosa, fibrosa, alba.

Ufus: Radix cordialis dicitur eumque in finem a Japonensibus cruda adhibetur. Sub cineribus affata amara est.

Herbaceous perennial, weakly rhizomatous with a woody tuberous rhizome; stems climbing slender extending to many metres, woody towards base, lacking lenticels; leaves glabrous, petiolate, ovate with cordate to truncate base, acuminate at the tip, not usually borne in clusters, 3-7cm (15cm); inflorescence of axillary panicles, profusely flowering, ochreolae and inflorescence axes usually puberulent; flowers borne in ochreate clusters, very small, white 2-3 x 1.2-1.7mm, outer 3 tepals winged; stamens 8, filaments not puberulent, stigma trifid, fimbriate; hermaphrodite; fruiting perianth greatly enlarged,

resembling that of R. japonica, but wings more abruptly decurrent.

There is also said to be an erect form known as F. multiflorum (Thunb.) Haraldson var. cilinerve (Nakai) J. Bailey Comb. Nov.

Open situations, edges of thickets and scrub land at altitudes of up to 2,500m

Distribution: China (Figure 2.3) Taiwan, Korea and Indochina (Steward 1930). It is introduced to Japan and the USSR, and its common use in the East as a tonic herb may well have masked its native distribution.

F. cilinodis (Michaux) Holub.

Type description: Michaux Fl. Bor. Am, 1:241 (1803)

CILINODE. P. minutissime puberulum : caule anguloso, prostrato aut scandente : stipulis subacutis, basi extrorsum serie ciliorum circumdatis : foliis cordatis; calycibus fructiferis apteris.

Obs. Affine *P. Convolvulo*.

HAB. in Canada.

Herbaceous perennial, stoloniferous, perennating by basal buds; stems scrambling (erect form also known) to 4m; leaves pubescent to nearly glabrous, petiolate, ovate sagittate upto 10x7.5cm; inflorescence of few-flowered axillary and terminal panicles; flowers small, white, outer 3 tepals not keeled or winged, stamens 8, styles 3 separate divergent, stigmas short fimbriate; hermaphrodite; outer 3 tepals of fruiting perianth becoming keeled; achene black, shining, exceeding the fruiting perianth. There are two extra varieties known, var. erecta, and the more glabrous var. laevigata Fern. found in the S. Appalachians.

Habit: Twining in dense masses on rocks and bushes in open country and woodland, var. erecta reaching 60cm.

Distribution: Eastern Canada and U.S.A.

Fallopia convolvulus (L.) Löve

Type description: Sp. Pl.:364 (1753)

Convolvulus. 24. POLYGONUM foliis cordatis, caule volubili,
 floribus planiusculis.
 Helxine caule volubili. *Fl. lapp.* 154. *Fl. succ.* 323.
Hort. cliff. 150. *Gron. virg.* 157. *Roy. lugdb.* 217.
 Convolvulus minor, semine triangulo. *Bauh. pin.* 294.
 Convolvulum nigrum. *Dod. pempt.* 396.
Habitat in Europæ agris. ☉

Annual; stems trailing or climbing to 1m; leaves ovate, acuminate, cordate-sagittate at base 2-6cm; inflorescence of axillary and terminal spikes with sessile fascicles in the leaf axils; outer 3 perianth segments wingless or with very small wings (var. *subalata* (Lej and Court) Kent is broadly winged); stigma 3-lobed capitate; fruiting perianth enlarged in fruit closely investing but not exceeding the achene; pedicel usually less than 2mm; achene dull black, minutely punctate.

Habit: A plant of waste ground and cultivated fields, found only as a weed throughout its range.

Distribution: Northern Circumpolar.

Fallopia dumetorum

Type description: Sp. Pl.:522 (1762)

dumetorum 26. POLYGONUM foliis cordatis, caule volubili lxxvi, floribus carnato-alatis.
 Fagopyrum praelongum dumetorum, seminibus alatis. duplici more dispositis. *Dill. app.* 65.
 Fagopyrum scandens altissimum dumetorum, seminibus tribus alis pellucidis, *Rupp. jen.* 99. *Hall. belv.* 173.
 Fagopyrum sylvaticum scandens, flore foliaceo. *Pont. anth.* 265.
 Fagopyrum majus scandens, *Paill. paris.* 52.
Habitat in Europæ australioris sylvis umbrosis. ☉.
 Folia cordata lobis basens rotundatis. Caulis non striatus. Panícula e racemo composito, alternatim bifaria.

Very similar to F. convolvulus; annual, stems climbing, slender to 3-4m; leaves ovate, acuminate, cordate at base; 3-6cm; inflorescence usually in unbranched spikes, or in sessile fascicles; flowers with the 3 outer perianth segments strongly winged, green edged with white, stigma 3-lobed capitate; fruiting perianth much enlarged in fruit; pedicels 3-6mm (longer than F. convolvulus); achene black and shining.

Habit: A scrambling plant of hedgerows and bushes. ..

Distribution: Europe, Russia, China, Korea, Japan, Himalyas, Afghanistan and Iran.

Fallopia cynanchoides (Hemsley) Haraldson

Type description: J. Linn. Soc. 26: 338 (1891)

17. Polygonum (§ Linaria) cynanchoides, *Hemsl.*, n. sp.

Herba perennis? volubilis, caulibus elongatis tenuiusculis teretibus retrorsim hispidulo-pilosis. *Folia* longe petiolata, papyracea, mollia, ferrugineo-pubescentia, late hastato-cordata, circumscriptione suborbicularia, usque ad 2½ poll. diametro, abrupte acuteque acuminata, lobis basilaribus etiam saepius breviter acuminatis, supra parce strigillosa, subtus dense pubescentia, venis immersis inconspicuis; petiolus graciliusculus subteres, saepius circiter pollicaris, retrorsim hispidulo-pilosus; ocreae parvae, inconspicuae, arcte appressae, breviter acuminatae vel fere truncatae? *Flores* minimi (bene evoluti non visi), glabri, laxe racemoso-spicati vel anguste paniculati, pedunculo axillari elongato gracili; perianthii segmenta 5, orbiculari-concava; stamina 5; stylus brevissime triidus. *Nux* glabra, trigona?

HERB.: Kuei (A. Henry!). Herb. Kew.

Very distinct in its twining habit, with soft, hairy, broadly cordate-hastate almost tricaudate leaves and slender inflorescence. In foliage and habit it strongly resembles some asclepiads, especially the genus *Cynanchum*.

Herbaceous perennial (?); stems slender, climbing; leaves ovate-sagittate, petiolate, densely tomentose on the underside, trichomes as in F. cilinodis; inflorescence of few flowered lax axillary and terminal panicles; flowers as F. cilinodis apparently not keeled; fruits not seen. Very little is known of this species, the only specimen that I have seen is the type.

Habit: Grassland and roadsides (Yang Young-Chang pers. comm.)

Distribution: Hupeh, Kweichow and Szechwan provinces of China (Figure 2.6)

Fallopia scandens (L.) Holub

Type description: Sp. Pl.: 364 (1753)

25. POLYGONUM foliis cordatis, caule volubili, floribus carinatis.
Fagopyrum scandens americanum maximum. Tournef. mlt. 511.
Fagopyrum scandens, caule rubente, semine nigro. Gron. virg. 44. Cold. noceb. 93.
- Fagotriticum volubile majus virginianum. Pluk. alm. 143. t. 177. f. 7.*
Fagopyrum scandens f. Volubilis nigra major, flore & fructu membranaceis compressis. Sloan. jama. 46. list. 1. p. 138. t. 90. f. 1.
- β. *Fagopyrum praelongum dumetorum, seminibus alatis duplici more dispositis. Dill. app. 60.*
Fagopyrum sylvaticum scandens, flore foliaceo. Pont. anth. 265.
Habitat in America. ☉
Flores plantae β. non vidi, at structura hujus est.

Annual (perennial Gleason 1963); stems climbing, slender, extending some metres; leaves glabrous, petiolate ovate cordate to slightly hastate, acuminate 4-13cm in length; inflorescence of axillary and terminal panicles; outer 3 perianth segments winged; fruiting perianth much enlarged, coarse wrinkled leathery appearance, quite large 10-16mm, pedicels long to 6mm, both shiny and minutely punctate achenes seen.

Habit: Moist woods thickets and roadsides (Gleason)

Distribution: North America, China, Japan and Russia.



Figure 2.1

Natural Distribution of *R.japonica* and *R.sachalinensis*

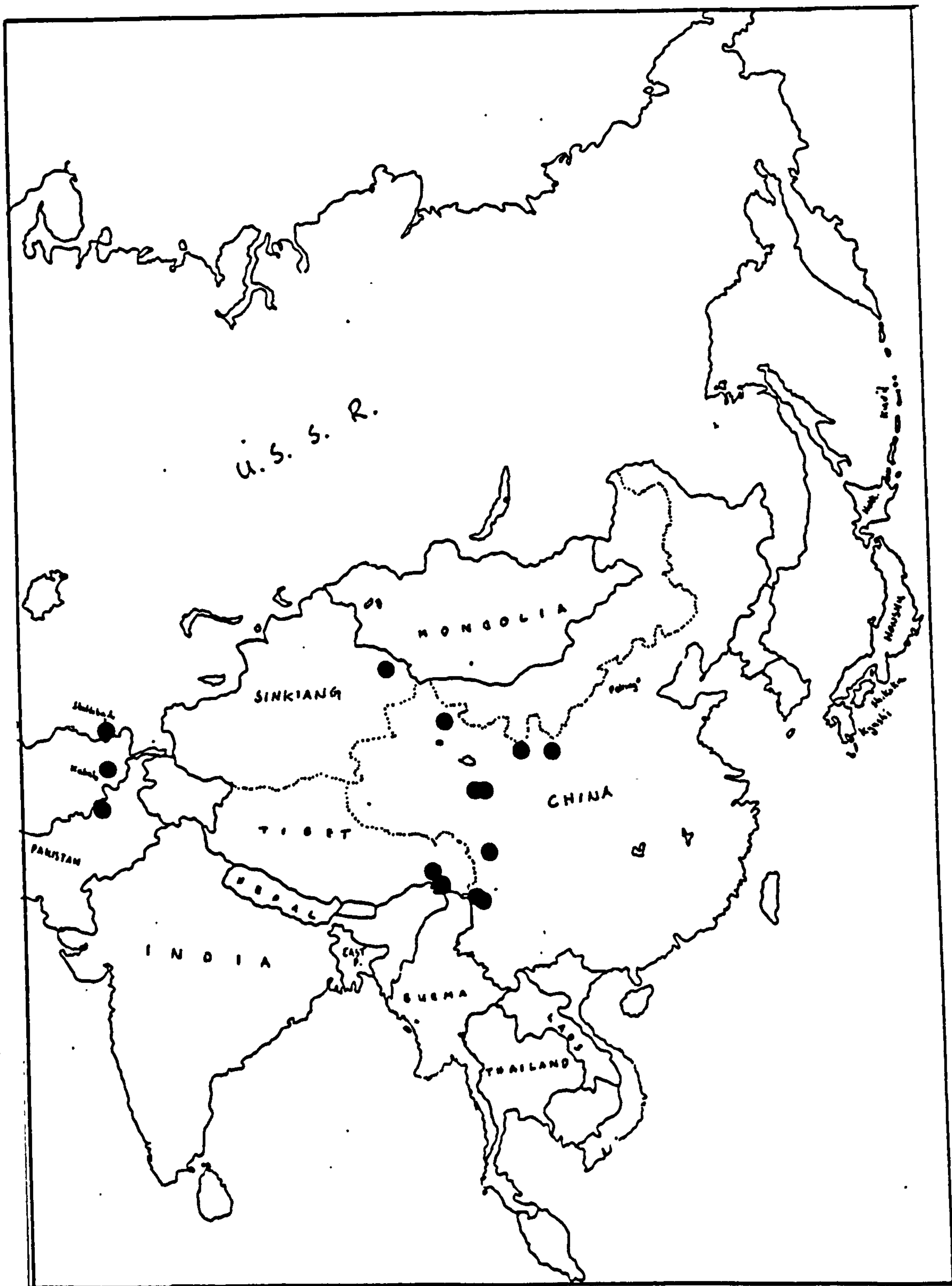


Figure 2.2

**Native Distribution of *F. baldschuanica*
(including *F. aubertii*)**

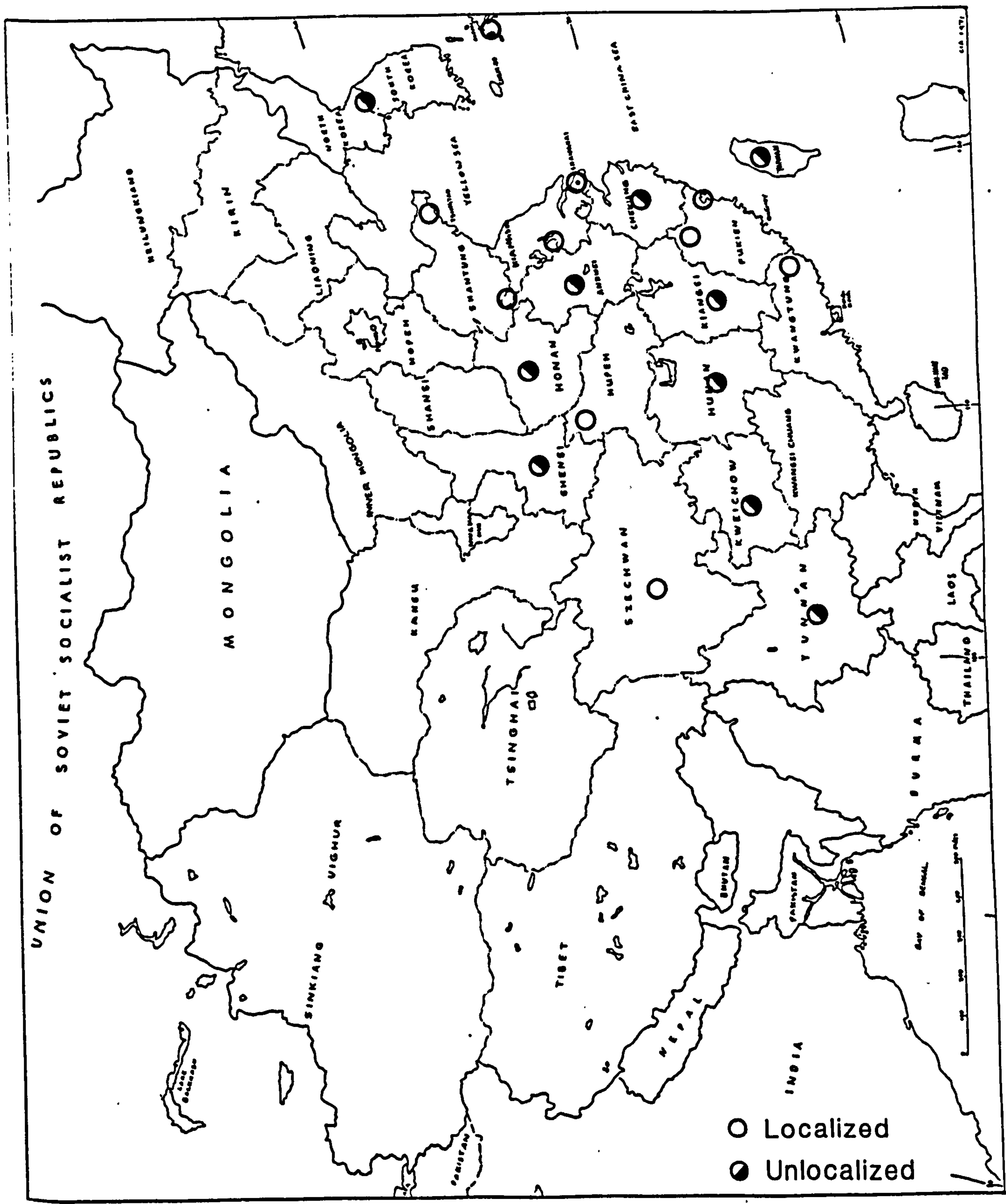


Figure 2.5

Distribution of *R. japonica* in East Asia (excluding Japan)

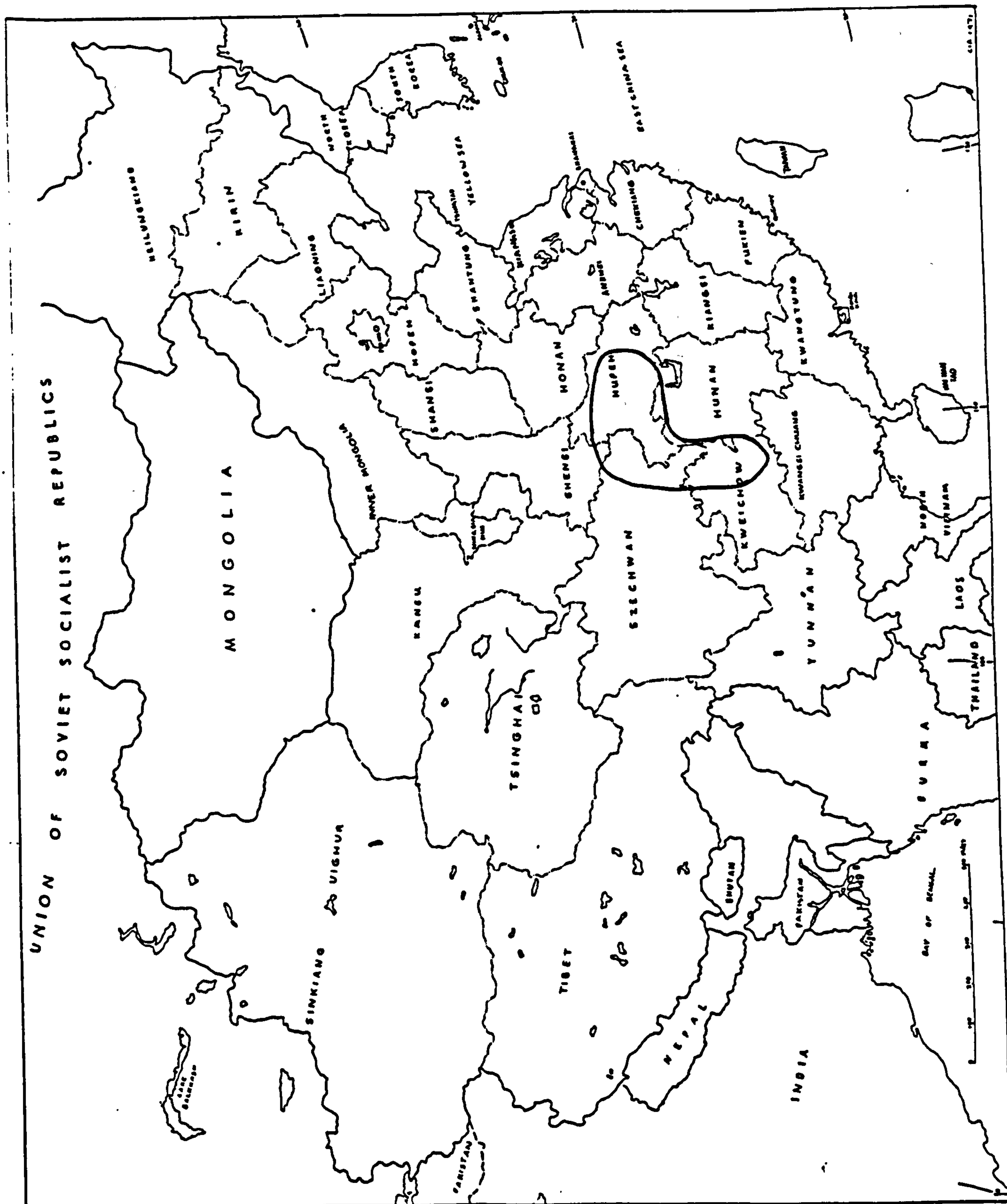


Figure 2.6

Distribution of *F. cynanchoides*

2.3 DISTRIBUTION

There can be little doubt that the centre of diversity for both Reynoutria and Fallopia is China and Japan where all taxa apart from F. cilinodis may be found. Indeed F. cilinodis bears much likeness to F. cyananchoides, but in the absence of living material or chromosome data concerning the latter it is difficult to comment further on their taxonomic relationship. This China-Eastern U.S.A. disjunct is not unique and is found in a number of genera which crossed from China to North America via the Bering Land Bridge; Li (1972) listed species from 14 different genera which have this same distribution. Whilst most are trees and shrubs some annual herbs including Polygonum species were mentioned. Stebbins (1950) stated that P. arifolium and P. sagittatum have a similar disjunct distribution and, since both are annuals, must have gone through more than 15 million generations without any significant evolutionary divergence.

Löve (1954), in the context of species or species pairs common to both Europe and North America, distinguished between those species that became geographically isolated first and then evolved differentially - vicariants; and those species that differentiated first and subsequently came to occupy different geographical regions - substitution taxa. These latter he equated with pairs of species differing in chromosome ploidy level. Unfortunately, lack of any cytological data on F. cyananchoides precludes any such analysis.

Figure 2.2 shows that F. baldschuanica appears to exhibit a disjunct distribution albeit of considerably less spectacular dimensions than that discussed above. Collections from the Chinese part of the range are often described as a separate taxon, F. aubertii (Henry) Holub. An examination of a range of such specimens from Russia and China reveals no good grounds for maintaining this specific distinction, although there is a tendency for the Chinese material to have somewhat smaller flowers and more papillose inflorescence axes-both incidently F. multiflora characters. It is probable that this apparently disjunct distribution is caused by lack of recording in the intervening territories. This is due to the notoriously inhospitable nature of these regions. These comprise the desolate province of Sinkiang and its borders with Mongolia and Russia; increased botanical surveying of the region in the future may well show this taxon to have a continuous distribution.

F. convolvulus is the most far-reaching of the taxa under discussion, having, according to Hulten (1971), a circumpolar distribution, albeit only as a weed throughout its range. The history of its early spread will probably never be known, but it is said to have been introduced to North America (Gleason, 1963), Japan (Ohwi 1965), Taiwan (Lai, 1976) and the Phillipines (Merrill 1926), the last within living memory. It is indeed tempting to conjecture that F. convolvulus (which is often tetraploid) evolved from F. scandens as a weed of cereal crops, its loss of wings perhaps making it a more effective seed contaminant. Perhaps this is another case of primitive man unwittingly

selecting for better cereals and better weeds simultaneously. Parallels with this are given by Stebbins (1950) in the case of the Crucifer Camelina sativa subsp. linicola Sch. et Sp.. This taxon is one of a group of weeds associated with flax cultivation, and which bear a number of morphological adaptations which ensure their incorporation into the flax seed harvest, but which have little adaptive value outside this niche.

F. multiflora (Figure 2.3) and R. japonica (Figures 2.1, 2.5) have more or less the same distribution, but with R. japonica extending further north-east into Japan, where F. multiflora is said to be an introduction. R. sachalinensis (Figure 2.1) has altogether a more easterly distribution, being restricted to Japan, Sakhalin, the Kuriles and Korea. ^{am} I am unsure whether this distribution extends to mainland Korea since the only localized reference to R. sachalinensis there relates to the Ullungdo Islands (Lee 1972), which lie between Korea and Japan. The distribution of R. sachalinensis is partly sympatric with that of R. japonica in Japan, whilst F. multiflora is sympatric with R. japonica in most of China.

2.4 MORPHOLOGY

2.4.1 THE POLYGONACEOUS FLOWER

According to Laubengayer (1937) the degree of homogeneity of the structure of the Polygonaceous flower is remarkable, considering the size of the family, and is indicative of a natural grouping. In the family as a whole the flowers are generally perfect but with a few dioecious taxa. There is some dispute over whether the trimerous or pentamerous flower is primitive. Although Laubengayer, in his study of flower vascularization, found that the apparently spiral 5-merous perianth found in Polygonum s. lat. represents the fusion of one of the inner tepals with one from the outer whorl. This is in agreement with Ronse de Craene (1986) who finds this more convincing than the tepal of a primitively pentamerous flower splitting into two units belonging to two different whorls to give rise to the two whorled hexamerous flower. The basic trimerous structure of the Polygonaceous flower is best illustrated by Rheum rhaponticum. Here there is an inner and outer whorl each of 3 tepals, an inner whorl of 3 stamens and an outer whorl of 3 pairs of stamens alternating with the inner stamens, and a fused 3-carpellary ovary bearing a single orthotropous ovule. The flower in Reynoutria and Fallopia is of the 5 tepaled sort, the outer three being generally winged; all taxa also have 8 stamens.

Despite the homogeneity of the Polygonaceous flower there exists plenty of variation in the various structures

associated with seed dispersal. In Polygonum virginianum L. for instance the stigmata become woody and barbed as an adaption to animal dispersal, whilst in Coccoloba and Polygonum molle D. Don the tepals become red and swollen in fruit to produce a berry-like structure. This contrasts with exposed nutlets produced in many species and with the winged persistent perianth that accompanies the nutlets in Reynoutria and Fallopia.

2.4.2 GROUP CHARACTERISTICS OF FALLOPIA AND REYNOUTRIA

Reynoutria and Fallopia have in common certain morphological characteristics which distinguish them to a certain extent from related taxa within the sub-family. Chief of these are the presence of a persistent winged perianth which can be found in all taxa except F. cilinodis and probably F. cynanchoides. The pollen type too was considered consistent enough by Hedburg for him to designate it Tiniaria type. This is described as tricolporate, spheroidal-subrolate, with narrow somewhat depressed furrows, usually extending more than 80% of the polar axis; pores small, elliptical, but sometimes extending laterally to form a ring in the endexine inside the equator; surface smooth to finely reticulate. A slightly aberrant type was found in F. dumetorum and F. convolvulus.

Another unifying feature though I do not know the extent of its presence in related genera, is that of extra-floral nectaries. It is perhaps significant that all 8 taxa

studied by Salisbury (1909) in his research into extra-floral nectaries are in the genera Fallopia or Reynoutria. It would certainly be of interest to know more about the distribution of extra-floral nectaries in Polygonum s. lat. and whether their presence is a correlate of taxonomic rather than physiological factors. Salisbury noted that there were no reports of insects visiting them, and supposed the extra-floral nectaries to be involved in water regulation during periods of rapid growth, and that they functioned as hydathodes. Significantly he noted that the occurrence of these nectaries in the one woody taxon (F. baldschuanica) is restricted to the rapidly elongating shoots, and that they are absent from the rosettes of leaves at the base of the annual shoots. This might be a good opportunity to ponder just what sort of woody perennial F. baldschuanica is. Its mode of growth, subject as it is to a massive surge of annual growth from every conceivable point in the spring, is not so unlike that of the Reynoutrias, with the woody stem replacing the woody rhizome. I cannot agree with Salisbury's comments about insect visitors to Reynoutria extra-floral nectaries since I have seen ants attend them on several occasions; moreover the exudate is quite sugary in taste. However, no-one who has ever broken a very young Reynoutria stem and seen the water pouring from it can doubt the turgor pressure involved, and that some device for water regulation would be advantageous.

Reynoutria and Fallopia also share, though not exclusively, the following characteristics: an inflorescence that is in panicles, simple spikes or axillary fascicles; a trifid style

with fimbriate stigma; or a fused style with a 3-lobed capitate stigma; and a trigonous nutlet enveloped in a brown or black pericarp. This pericarp is generally quite thin in Reynoutria and F. multiflora, but is very thick in F. baldschuanica and F. convolvulus and is implicated in the regulation of germination (Justice 1941).

Reynoutria Houtt.

Morphologically this genus is rather uniform, consisting entirely of erect herbaceous perennials with woody underground rhizomes. Such variation as exists is restricted chiefly to the size and habit of the plant, the leaf shape and the trichome types. The genus has an entirely Asiatic distribution and does not cross the Himalayas to India.

Male-fertile and male-sterile flowers are usually borne on separate plants, the stigmas on the male-sterile plants being trifid and fimbriate. The stigmas on the male plants vary from small undifferentiated knobs to well formed extended fimbriate structures, with all intermediate forms. In all taxa the nutlets are enclosed in a persistent, much enlarged, winged fruiting perianth. It is not known if this is an aid to seed dispersal, though it must be noted that the fruits are usually well attached to the dead stems, but by winter the nutlets have often been shed from the winged perianth, although animal or bird predation cannot be entirely ruled out.

Fallopia Adans.

In contrast with Reynoutria, Fallopia is morphologically a much more diverse genus. This stems mainly from the four different life forms that it embraces: woody perennial, herbaceous rhizomatous perennial, hemicryptophyte and annual. These different life forms are reflected to some extent by the sectional treatment accorded them by Haraldson (1978) on the basis stem and petiole anatomy and morphology:

Fallopia sect. Pleuropterus (Turcz.) Haraldson.

comprises the woody perennial F. baldschuanica and the herbaceous perennial F. multiflora. The latter being the only rhizomatous species in the genus. Both taxa have the winged perianth in flower and fruit. F. multiflora has a fimbriate stigma like that of Reynoutria, whilst F. baldschuanica has the trifid capitate stigma that is characteristic of the annual Fallopia taxa.

Incidentally the distinction between erect and climbing gets a little muddled at this point since there is said to be an erect variety of F. multiflora. This is also distinguished by its longer leaf blades, with hairs on the underside (Steward 1930). Specimens in the herbarium at Kew collected in Korea support this distinction (though not the point about the hairs); their dimensions (stems up to 1.3m long but only 4mm wide) suggest that support would be required, and that these plants would grow through rather than over the surrounding vegetation. Another possibility is that these erect plants are really hybrids between R. japonica

and F. multiflora though in the absence of living material this would be impossible to prove.

Fallopia sect. Parogonum Haraldson

Contains two taxa, F. cilinodis and F. cynanchoides.

Description of the morphology and life-form will be mostly restricted to F. cilinodis, since I have been unable to obtain live material of F. cynanchoides; little is written in the literature about it, and the herbarium material that I have examined contained only immature flowers.

Although both were described as hapaxanthic by Haraldson (1978), F. cilinodis is certainly a perennial, perennating by means of buds at the base of the current years growth and spreading by means of stolons. On the basis of its considerable morphological similarity with F. cynanchoides I suspect that this may also turn out to be a perennial. Section Parogonum differs in a number of ways from the other Fallopia sections, including its curious disjunct distribution, F. cilinodis being found in North America, and F. cynanchoides in Central China. The flower of F. cilinodis is unusual for a Fallopia taxon in that when open it is neither keeled nor winged, but the outer perianth segments do become keeled in fruit. The fruiting perianth is also rather distinctive in its not fully covering the mature nutlet. A link with the plants of erect habit in Reynoutria is provided by var. erecta, said by Gleason (1963) to have stouter red stems and grow in more open situations.

Fallopia sect. Fallopia Adans.

Comprises the annuals F. scandens, F. dumetorum and F. convolvulus. The distinction between F. scandens and F. dumetorum is rather tenuous, and some American authors such as Gleason (1952) regard the latter as a variety of F. scandens. Incidentally this treatment follows the precedent set by Linnaeus (1753), who classified F. dumetorum as variety B of F. scandens. The taxa in this section all possess 3 lobed capitate stigmas and winged or keeled perianths. F. convolvulus is distinguished by its secondary loss of perianth wings, although narrow wings are sometimes found and the variety F. convolvulus var. subalata is broadly winged. A further distinct adaptation of F. convolvulus is that of very reduced anther size (32 pollen grains per locule fide (Graham and Wood 1965)) with the anthers growing over the style to dehisce before the flowers open. This presumably points to the development of a cleistogamous inbreeding system (see Plate 2.7c)

2.4.3 CUTICULAR STUDIES

Conolly (unpublished), during the course of her research into Reynoutria species in the British Isles, found that R. japonica and R. sachalinensis exhibited distinct differences in the trichome type and the degree of cuticular ornamentation. Haraldson (1978) presented results which suggested that Reynoutria and Fallopia might be distinguished on the basis of trichome types. In order to increase the range of Reynoutria specimens examined, to include some of known wild origin, and to extend such observations to the genus Fallopia, cuticular preparations from a number of taxa were subjected to light-microscopical and S.E.M. investigation. In spite of the spectacular clarity and detail revealed by the scanning electron microscope, it was still essential to examine comparable material by light microscopy, since S.E.M. only recorded surface details, and in the case of trichome cell number and shape of epidermal cells light microscopy was indispensable.

2.4.3₁ Materials and Methods

Stace (1965) stressed the fundamental importance in any cuticular studies of the age of the leaf and the environment in which it is grown, since both factors can have a profound effect on epidermal cell shape and the degree of pubescence. Accordingly where possible material was grown together outdoors, only fully expanded mature leaves were used, and the material studied was taken from a standard area on the leaf (Figure 2.1).

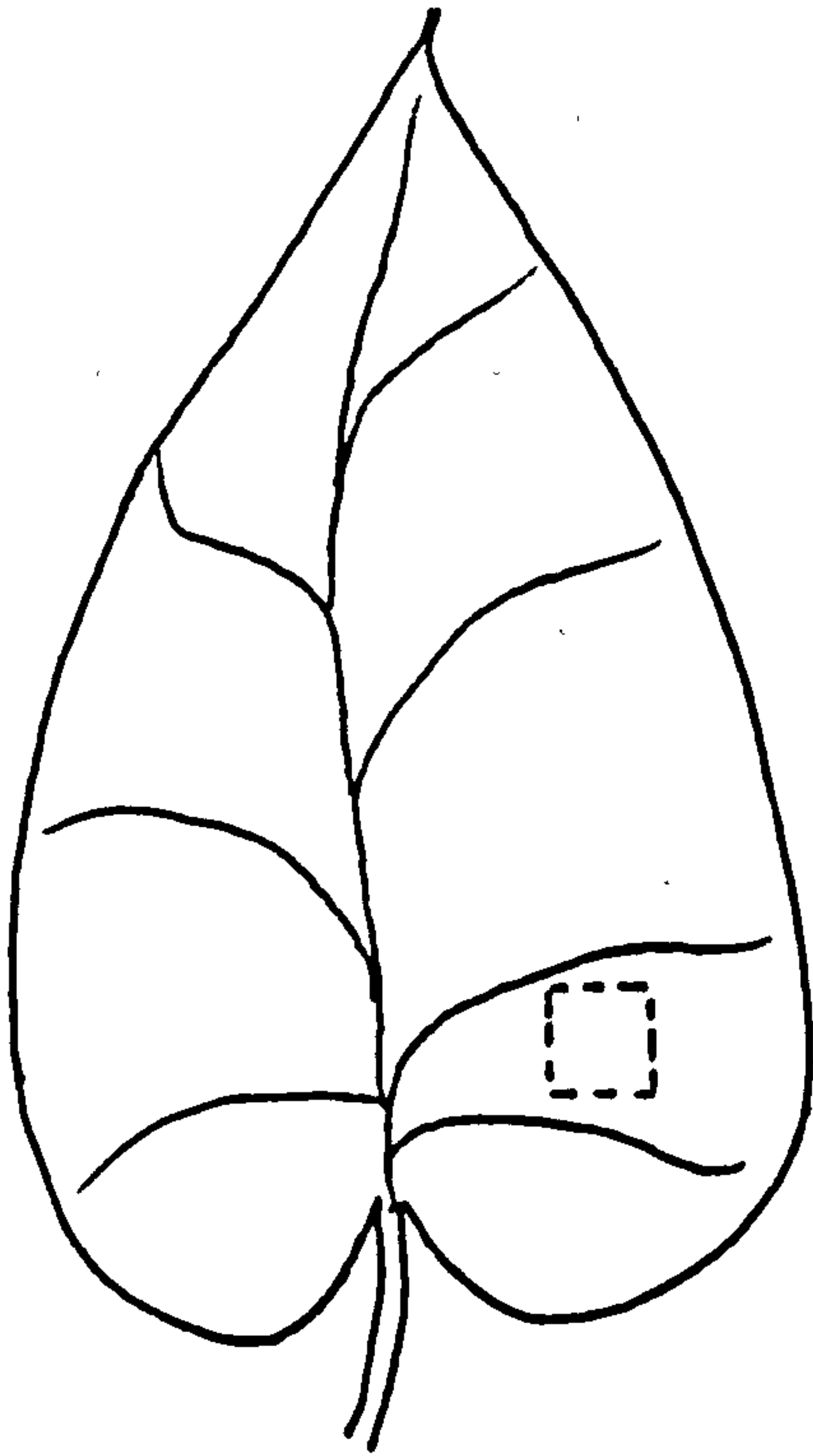


Figure 2.1 showing area of leaf used for cuticular studies.

In all cases only the lower epidermal surface was examined, since a preliminary examination of the upper epidermis had shown it to exhibit little variation between taxa. Furthermore, trichomes tended to be restricted to the lower leaf surface, although fugaceous hairs have been observed on the upper surface of emerging R. sachalinensis leaves. In Reynoutria taxa it was particularly important to make sure that the largest basal leaves available were used. since these exhibited the fullest range of trichomes, and the occurrence of trichomes on the smaller leaves was somewhat sporadic.

A) Light Microscopy

I) With fresh material epidermal peels were obtained by making a fine cut parallel with the leaf surface with a razor and peeling back with fine forceps. Epidermal peels were mounted in 50% glycerol and sealed with rubber solution. The disadvantages of this method was that it was difficult to obtain epidermis from the chosen collection areas and that in some heavily cutinized specimens peels were very difficult and only small areas adjacent to the veins could be obtained. Good trichome preparations were obtained by this method.

II) To overcome the problems of I), and to have a technique suitable for herbarium material, the following procedure was employed. Squares of leaf were boiled in 88% lactic acid for 30 minutes, then placed abaxial side down on a glass plate in 88% lactic acid. The adaxial surface and intervening tissue were carefully scraped away with a sharp scalpel blade. Scraped epidermises were then washed in distilled water, mounted in 50% glycerol and the cover-slips sealed with rubber solution.

Measurements were made using an eye piece graticule at x16 or x40 on a Zeiss standard microscope. Photographs were taken on a large Zeiss Universal microscope using FP4 film at 200ASA with microphen development.

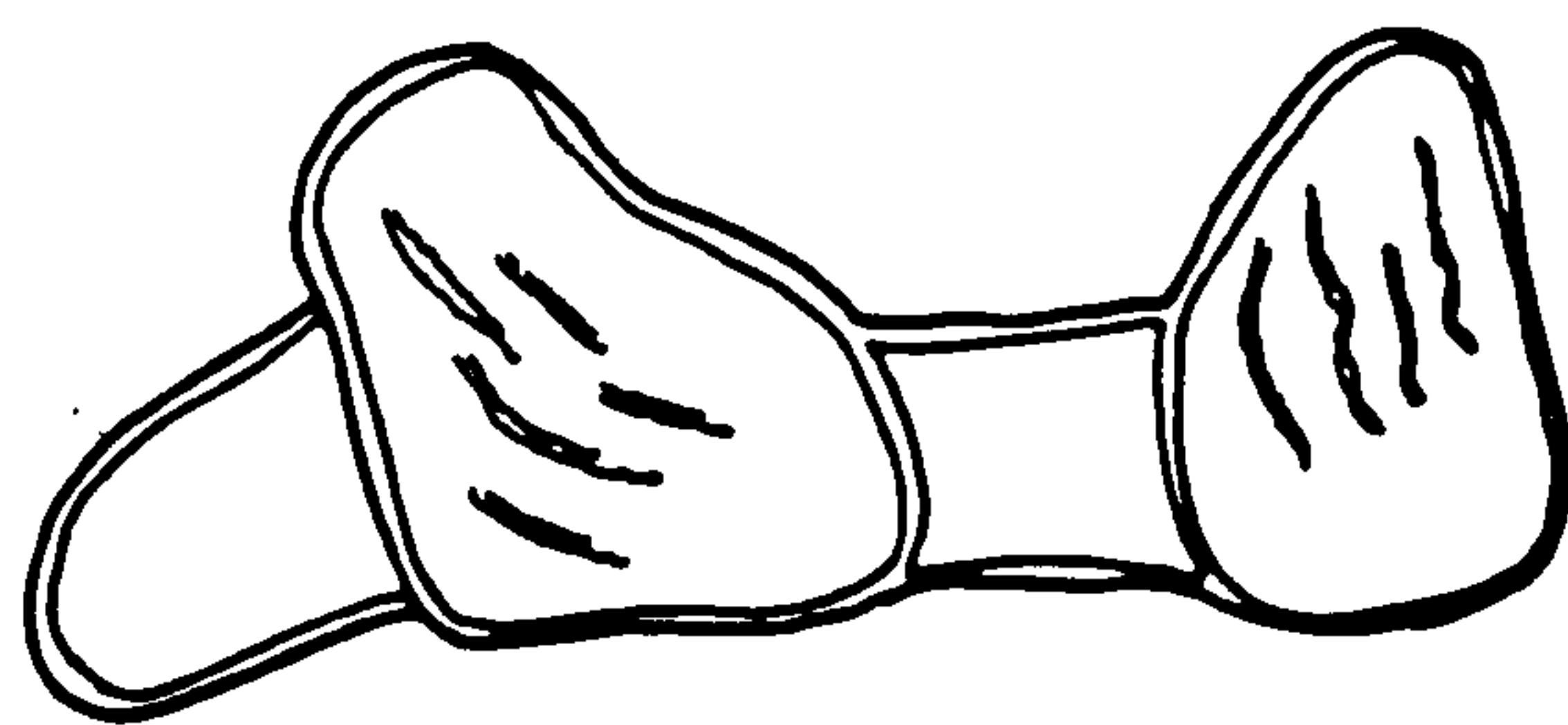
B) Scanning Electron Microscopy

Leaf material was taken through an ethyl alcohol dehydration series and into amyl alcohol. It was then critically-point dried in a Samdri 780, and coated with gold palladium in a Polaron ES400 cool sputter coater (-Ca 20 μ m thick) for 90 seconds. Specimens were examined on a Cambridge S100 Scanning electron microscope at 25Kv and later 15Kv, the lower Kv being preferable as this reduced 'charging' of the specimens. Photographs were taken using Ilford Pan F film with microphen development.

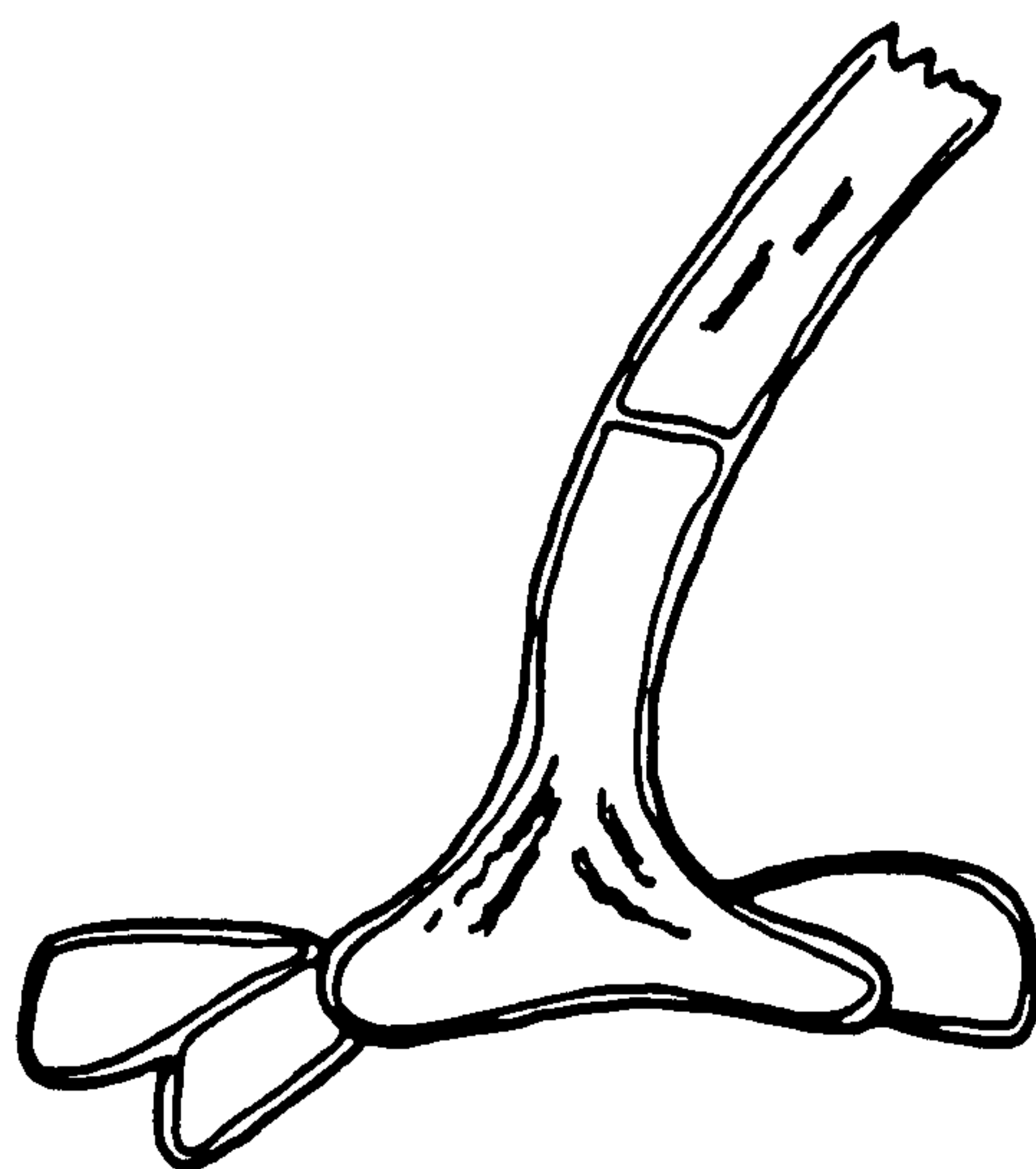
2.4.3_{ii} Results

Three broad types of non-glandular trichome were found to be present in the specimens examined (Figure 2.7). The simplest of these is type A, which is little more than a single swollen oval epidermal cell protruding above the surrounding cells and rather more striate than them. This type was the most commonly found and was seen to best advantage with the S.E.M. (e.g. Plate 2.4e). Type B is a uniseriate multicellular hair comprising 4-20 cells and up to 1.6mm in length (Plate 2.3b). These trichomes are not very heavily striate and tended to collapse very easily, invariably so on herbarium specimens (Plate 2.3c). Type C hairs are stiff and unicellular with a papillate surface, and arise from a base without accessory cells (Plates 2.2c,d)

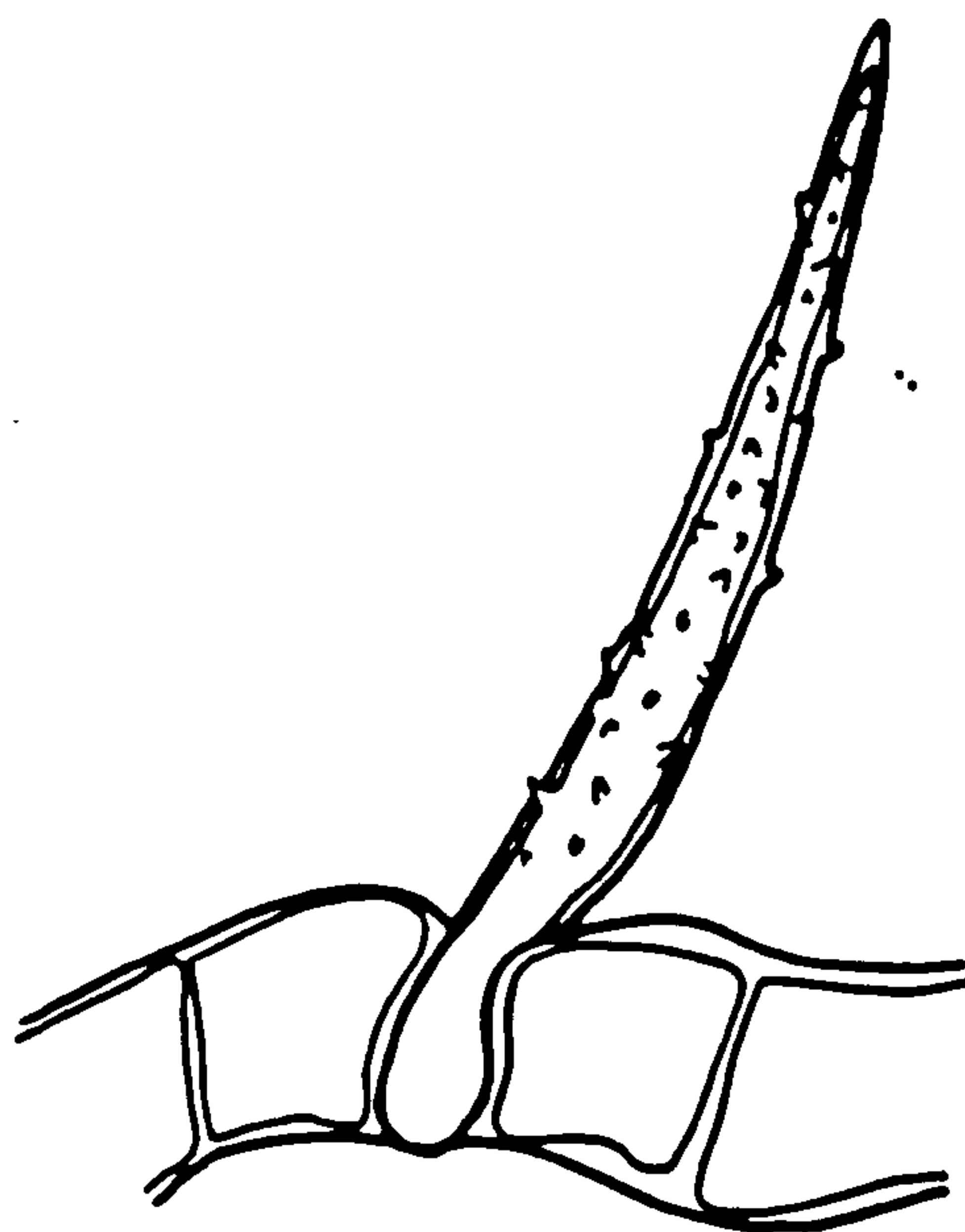
The epidermal cell-wall outlines of the lower leaf surface



Type A



Type B



Type C

Figure 2.7 *Reynoutria* and *Fallopia* trichome types

are generally rather undulate, the degree of cuticular ornamentation varying from just a few striae in the region of a stoma (Plate 2.4f) to the heavily folded cuticles that obscure the epidermal cell outline (Plate 2.4a,b).

Details of the specimens examined, trichome types present and the degree of cuticular ornamentation are given in Table 2.1. Also shown are some sample measurements of the length and breadth of epidermal cells, and some mean measurements of stomatal length and breadth.

It may be seen from Table 2.1 that type B hairs are restricted to R. sachalinensis, including material of known wild origin and also type material. Type C hairs are restricted to F. cynanchoides and F. cilinodis, whilst type A hairs are more or less universally distributed, through the taxa, with the exception of the last two. This fully confirms and extends the unpublished observations of Conolly, as do the cuticular surface results. Incidentally whilst I refer to cuticular patterning as striation, it seems clear that this is the result of folding of the cuticular layer.

In R. sachalinensis this folding up of the cuticle is such that in some specimens (Plate 2.4a) the detail of the epidermal cells is obscured. This type of patterning is also present in F. cilinodis and F. cynanchoides, but in these it is less extreme and is clearly limited to the epidermal cell outline (Plate 2.2f). In R. japonica, F. multiflora and F. baldschuanica striation is usually

Species	Collection No.	Trichome type	Degree of cuticular striation	Specimen epid. cell length x breadth	Stomatal length and breadth	Remarks
<u>F. baldschuancia</u>	P163	A	±	79.4x44.6	32.98x25.54	
<u>F. baldschuancia</u>	P151			115.8x57.04	36x22	
<u>F. baldschuancia</u>	P98	A	±	69x52	32x28	
<u>F. multiflora</u>	P100			61x25	28x23	
<u>F. multiflora</u>	P162	A	-	110x52	26x21	
<u>F. cilinodis</u>	P148	C	++	71x50	26x18	
<u>F. cynanchoides</u>		C	++	*	*	
<u>R. sachalinensis</u>	P55	B	+++	64x40	38x30	very heavily striate
<u>R. sachalinensis</u>	P56	B	++	89x61	36x29	
<u>R. sachalinensis</u>	P57	B	+++	76x50	*	
<u>R. sachalinensis</u>	P61	A,B	++	74x50	*	
<u>R. sachalinensis</u>	P62	±A,B	+++	*	32x32	
<u>R. sachalinensis</u>	P115	B	+++	*	29x26	
<u>R. japonica</u>	P12	A	±	106x60	39x33	
<u>R. japonica</u>	P105a	A	±	94x67	33x27	
<u>R. japonica</u>	P113	A	±	172x91	42x28	
<u>R. japonica</u>	P114b	A	-	74x37	32x28	
<u>R. japonica</u>	P166	A	±	107x52	37x27	
<u>R. japonica</u> var. <u>compacta</u>	P2	A	±	96x55	33x26	* data not available

TABLE 2.1 OCCURRENCE OF TRICHOME AND CUTICLE TYPES

restricted to the cells surrounding the stomata (Plate 2.4f), where it may possibly have some structural function. The cuticle overlying cells not adjacent to the stomata is usually without any ornamentation (Plate 2.4d), although faint striae can sometimes be found (Plate 2.1e).

The few specimen measurements of the length and breadth of the often asymmetric epidermal cells serve only to illustrate the considerable variation that exists.

2.4.3_{iii} Discussion

These results illustrate the point that whilst some cuticular characters may clearly distinguish certain species, in other taxa the same or different cuticular features are of no taxonomic value. R. japonica may be distinguished from R. sachalinensis on two clear characters, trichome type and degree of cuticular ornamentation. F. cilinodis and F. cynanchoides may be distinguished from all other taxa but not from each other on the basis of cuticular ornamentation and on their unique trichome type. Indeed so unusual is this type of trichome that Haraldson (1978) found it to occur only in these two taxa out of all those examined in the sub-family Polygonoideae. F. baldschuanica, F. multiflora and R. japonica, on the other hand, cannot be distinguished from each other by the use of these cuticular characters, although the outline of epidermal cells in R. japonica is much thicker than in the other taxa, and might be of some use in distinguishing them.

Haraldson's (1978) trichome types VII and IX more or less correspond with my types A and B but, as she recorded their presence at the generic level only, one does not know if she was able to make any distinction between the Reynoutria taxa. She also recorded the presence of glandular trichomes (Plate 2.3d), but their ubiquitous occurrence in Fallopia and Reynoutria makes them of little taxonomic value.

These cuticular characters are of particular value when it comes to the examination of herbarium specimens, since these usually only have the smaller leaves associated with the inflorescences which do not have the characteristic shape of the lower leaves.

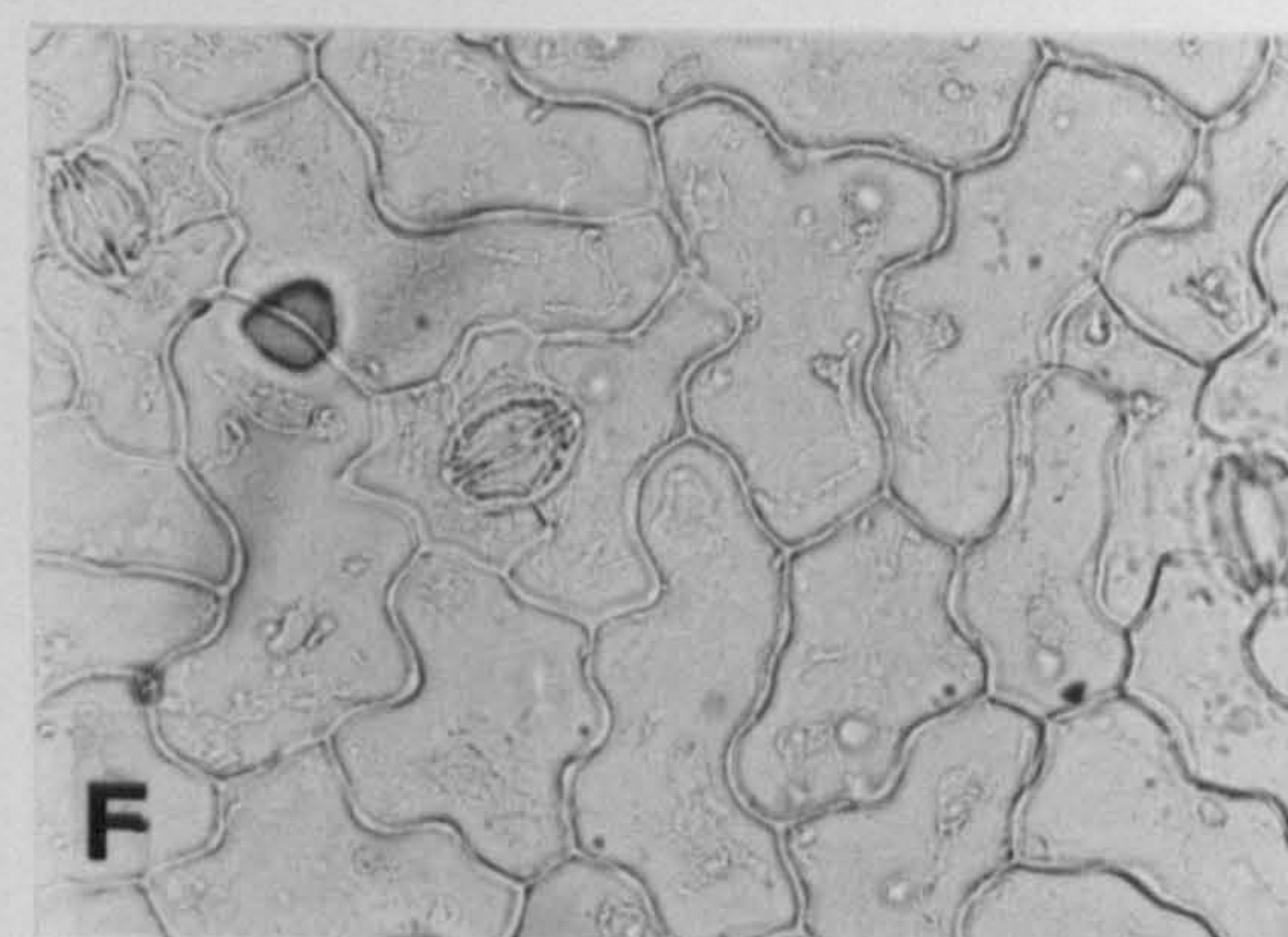
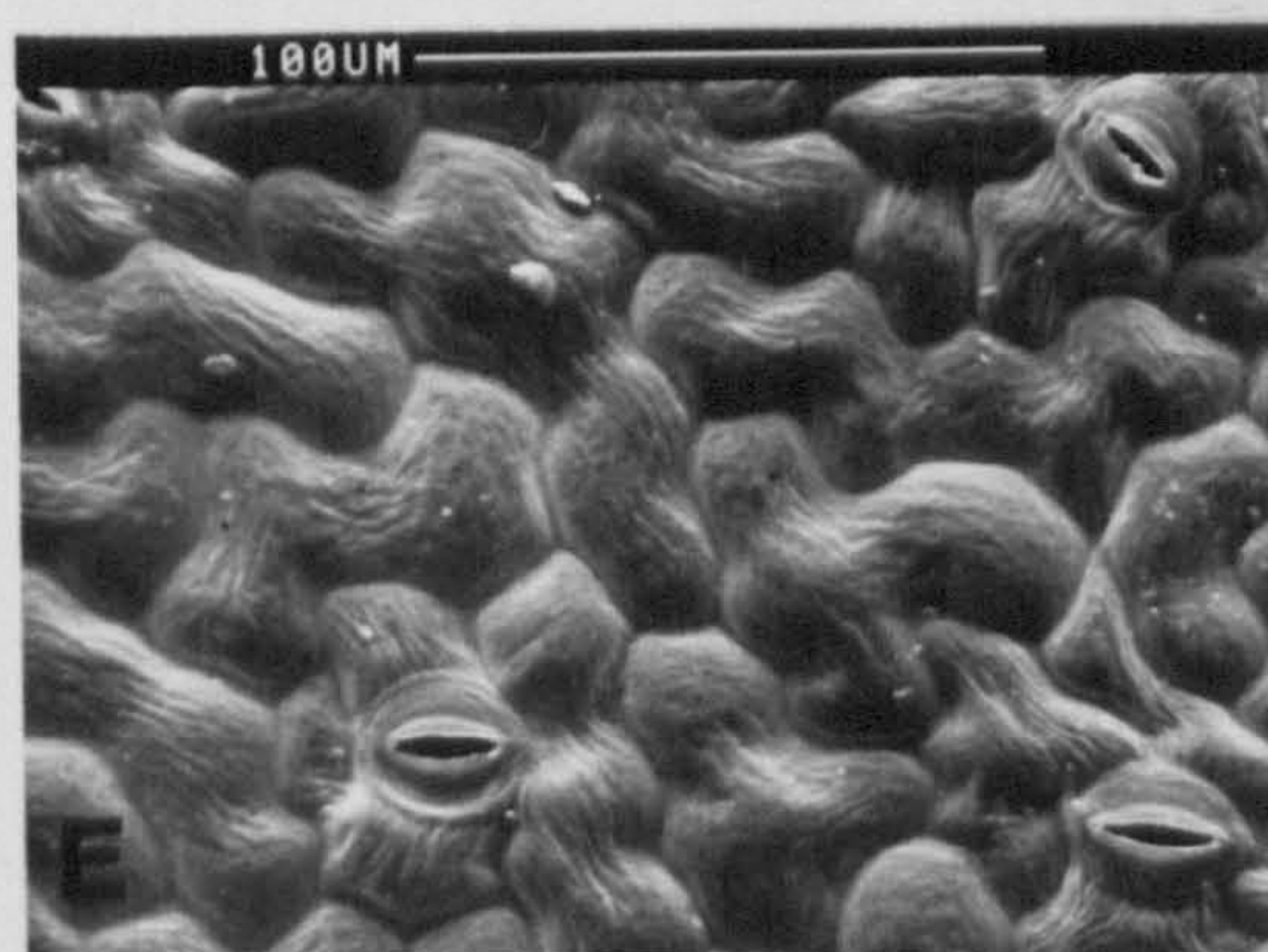
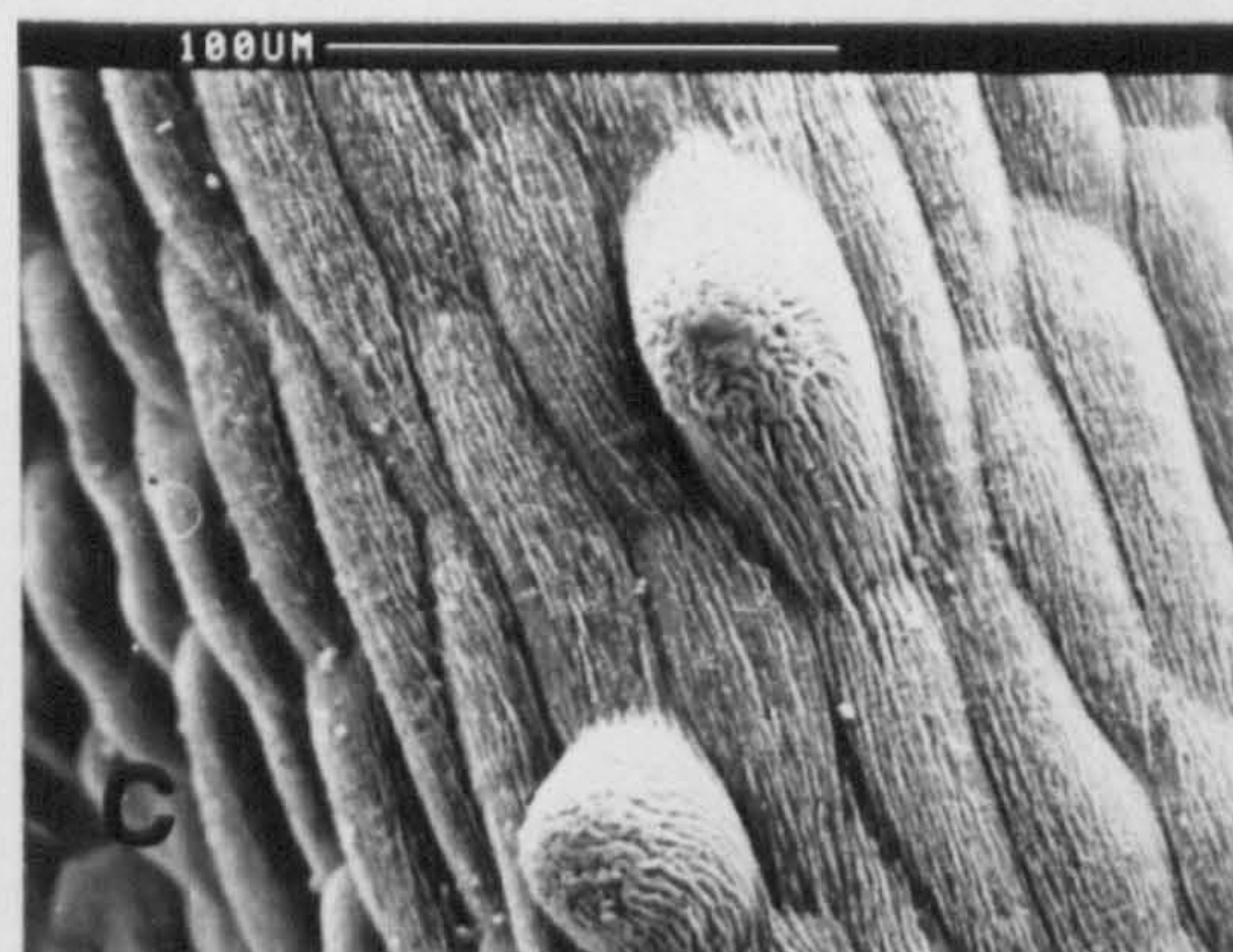
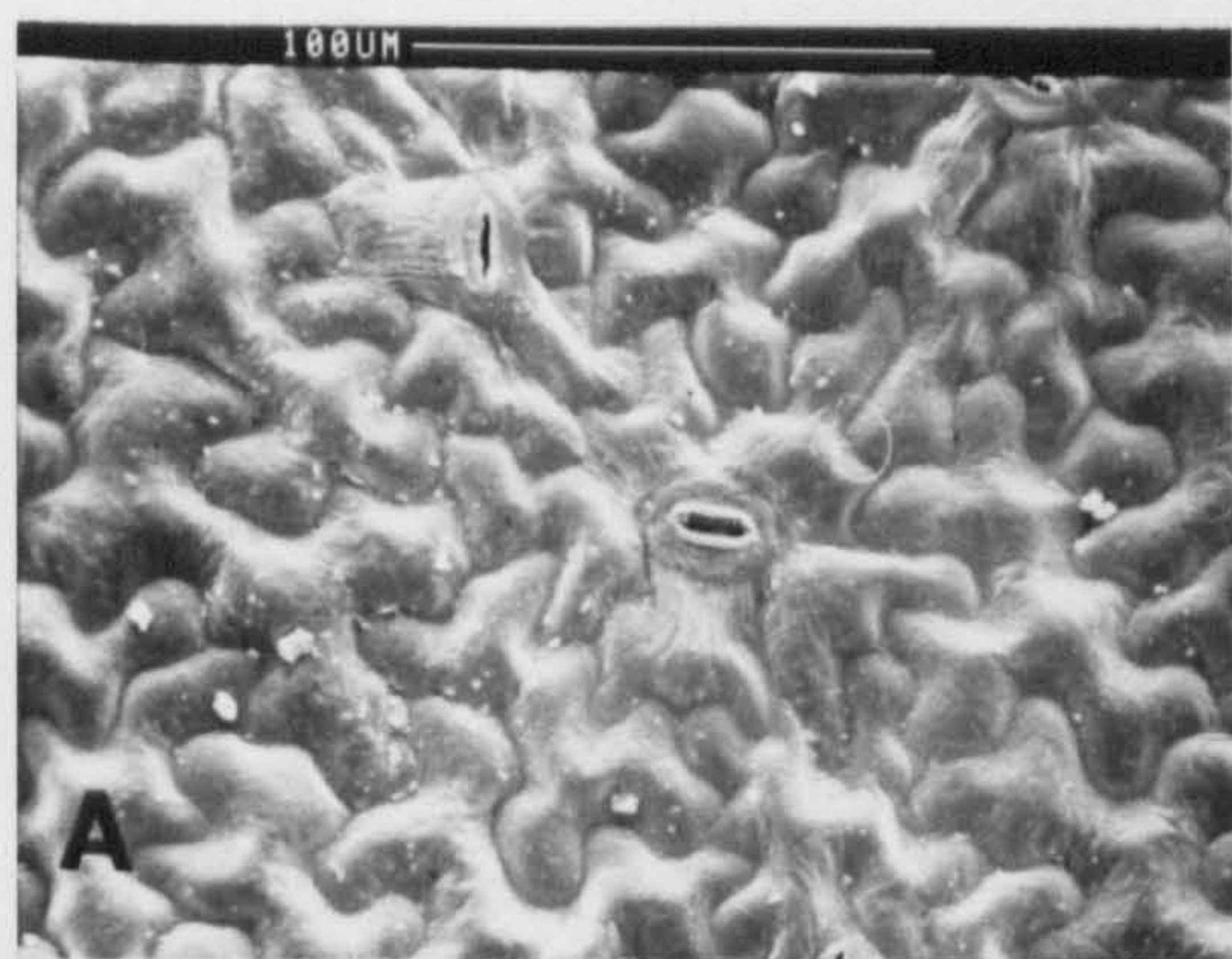


Plate 2.1 Diploid epidermal and cuticular morphology

A,B *F. baldschuanica* P 151 epidermis by SEM and light microscopy

C D P 151 trichomes

E.F *F. multiflora* P162 by SEM and light microscopy

NB Epidermal preparations X 312 and trichomes X 160 unless otherwise indicated

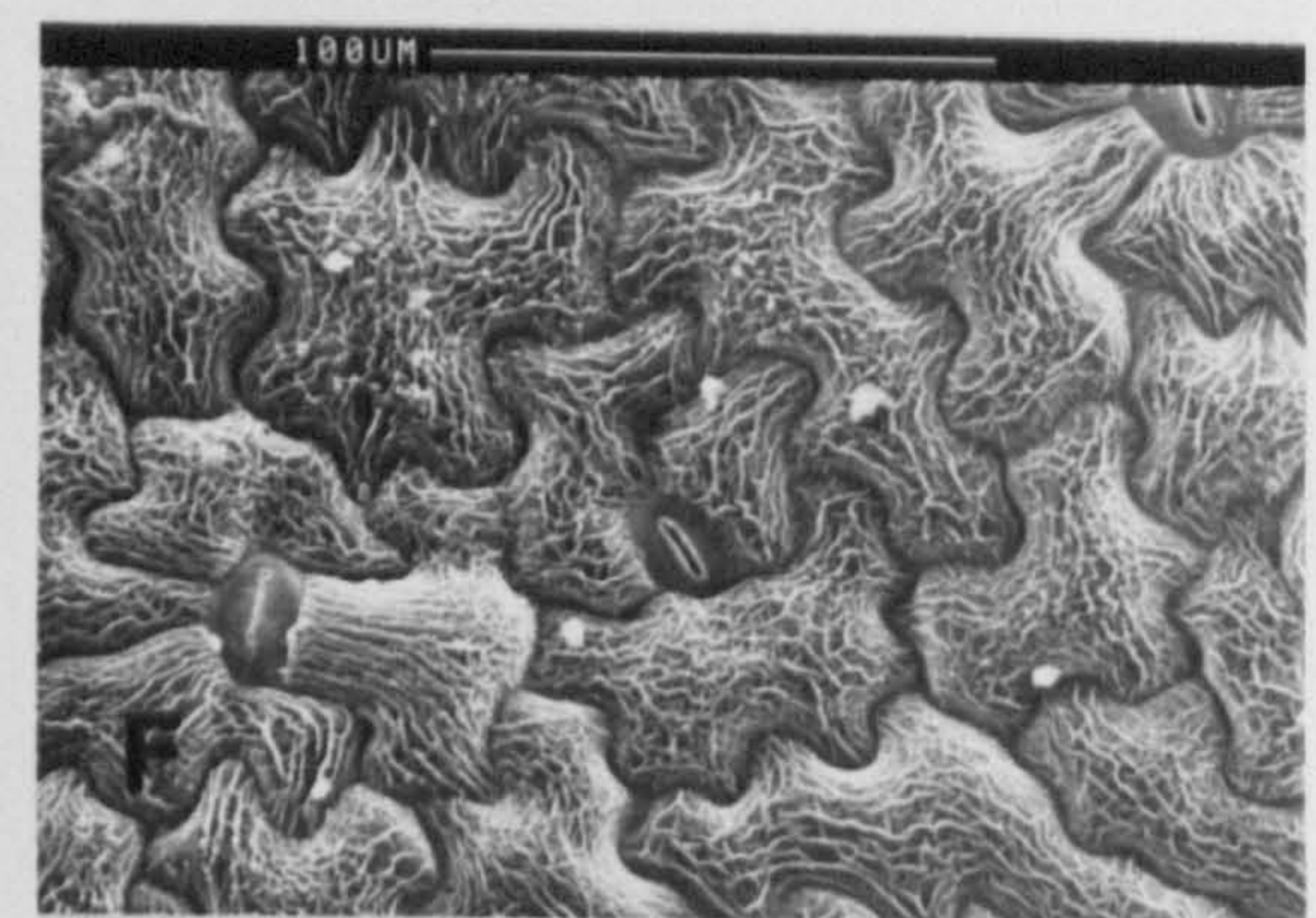
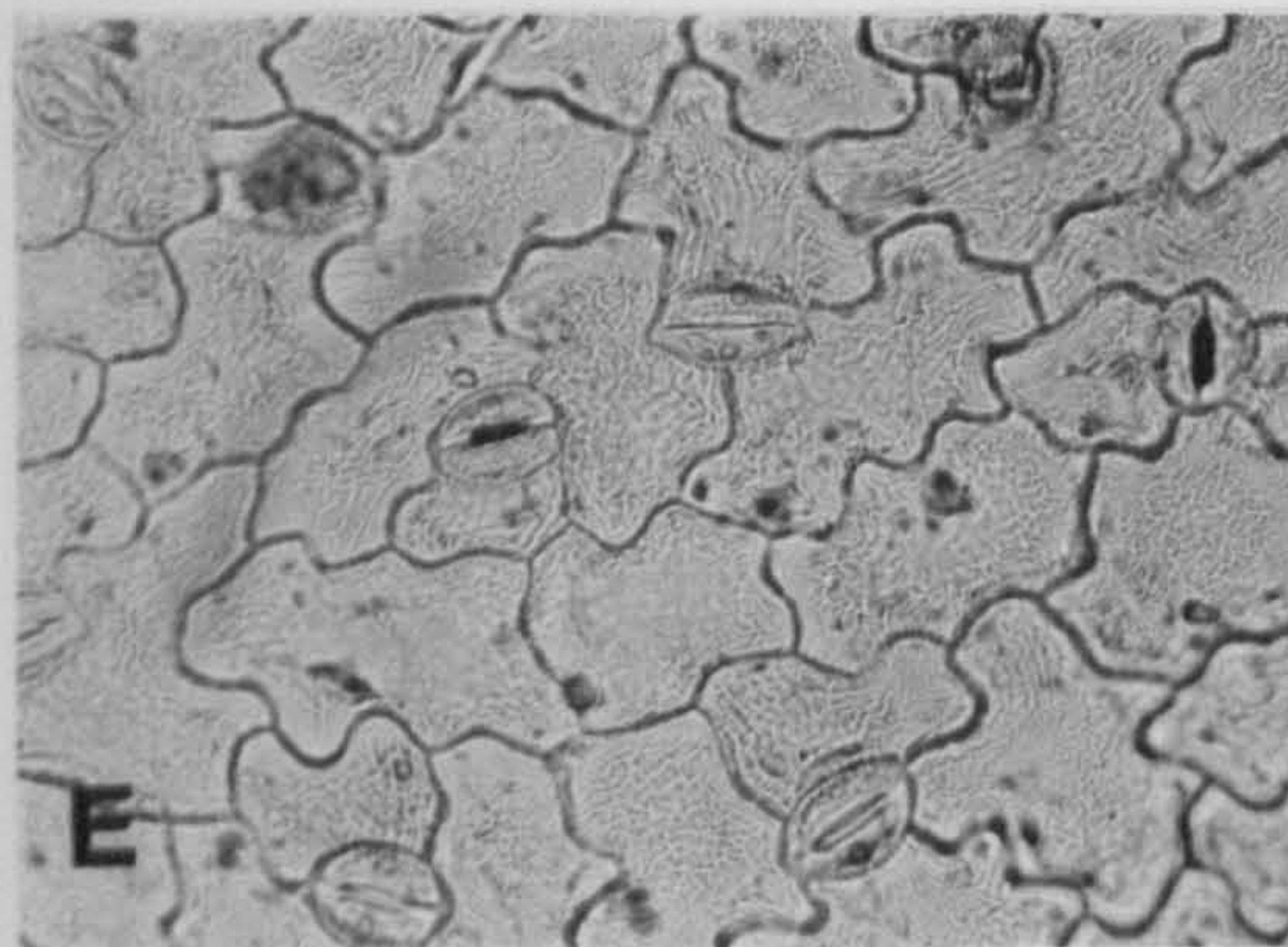
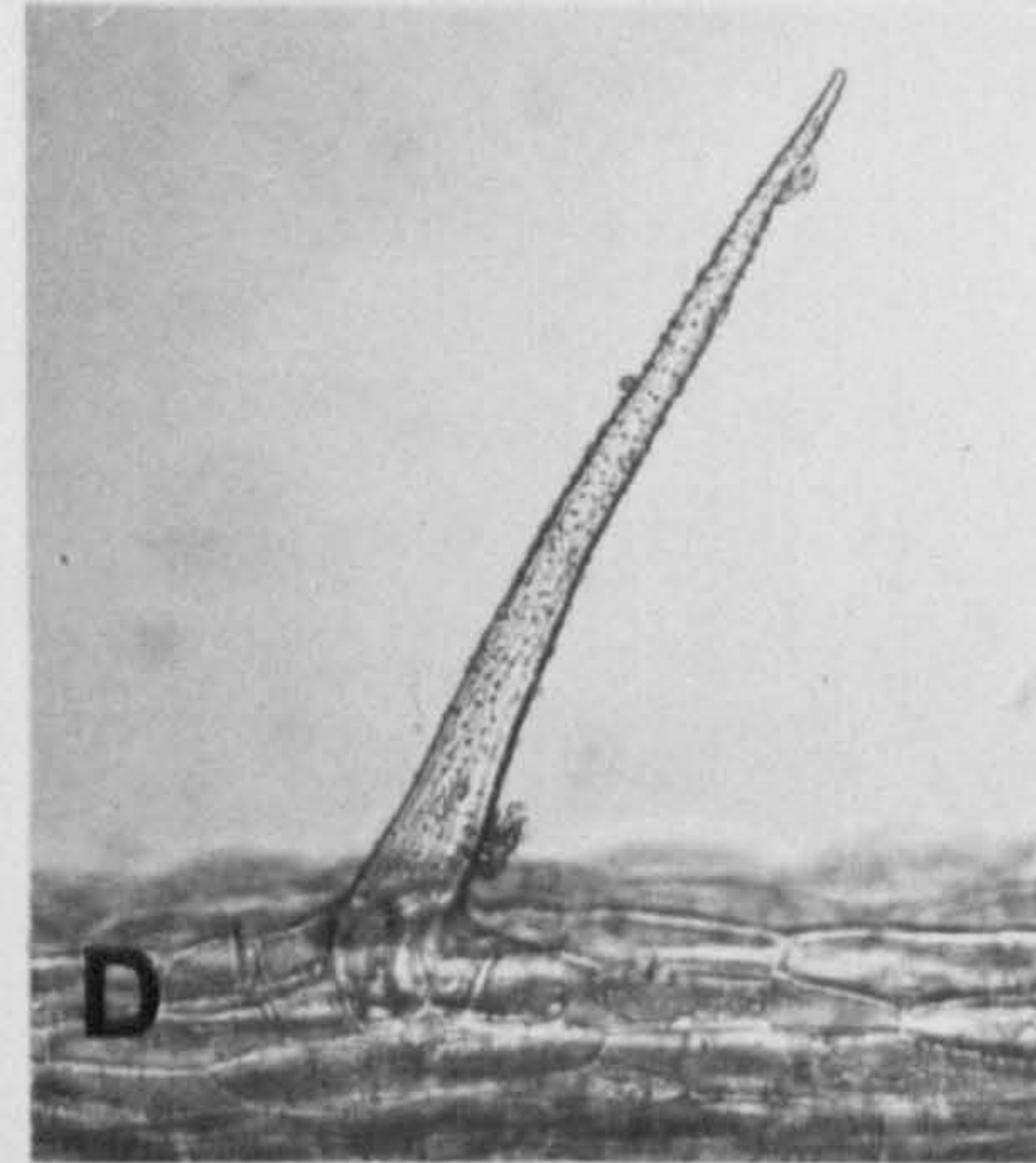
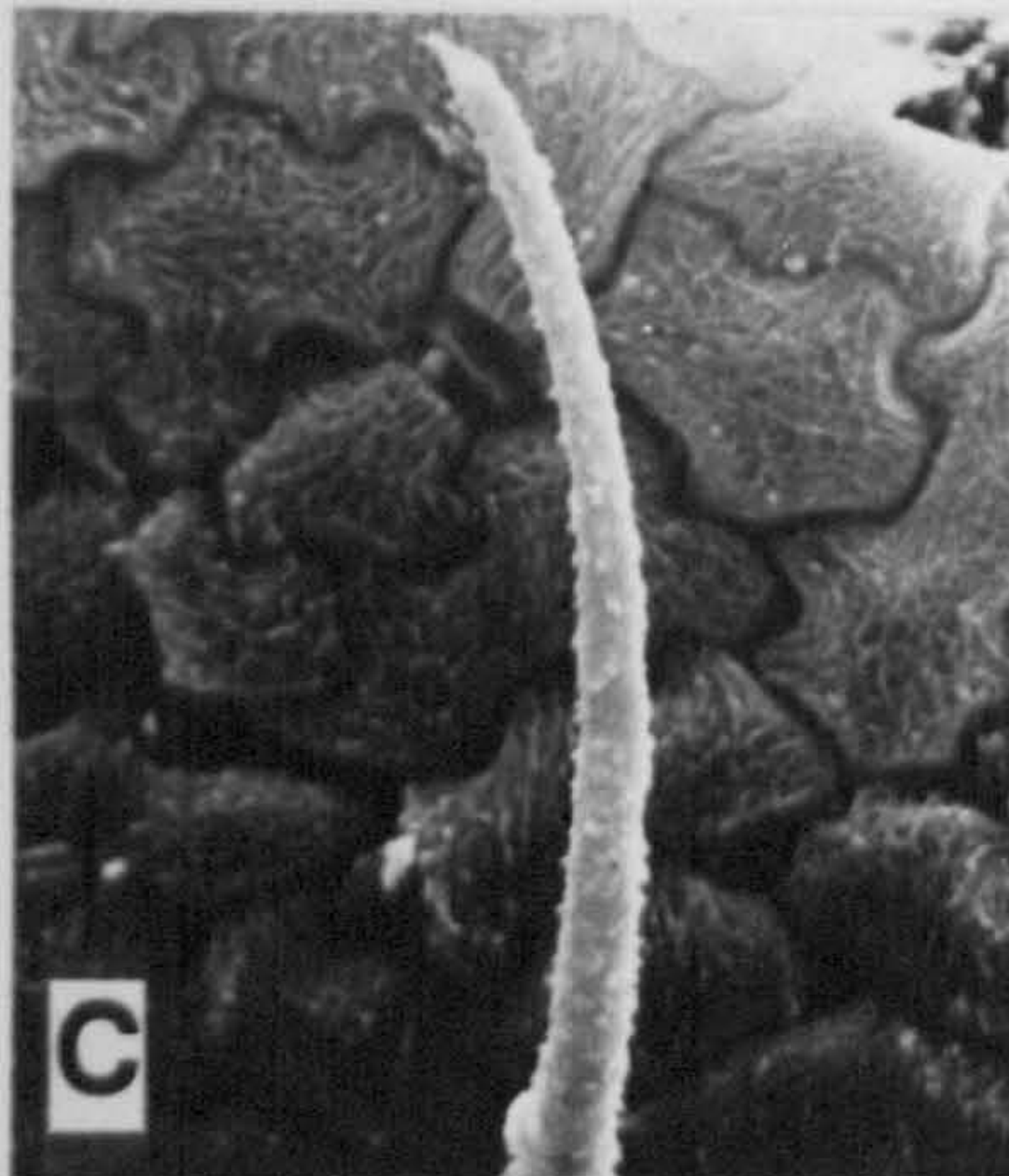
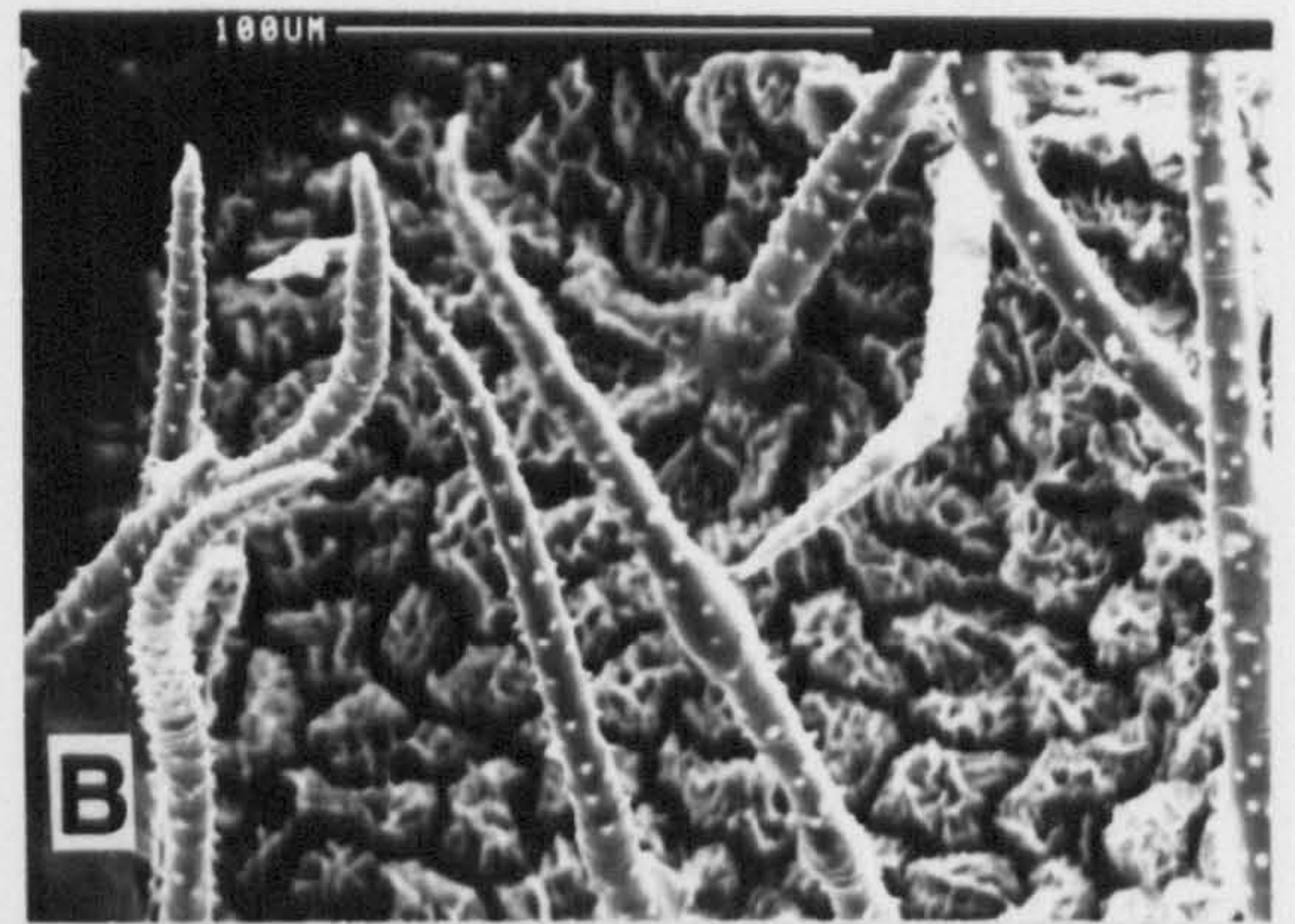
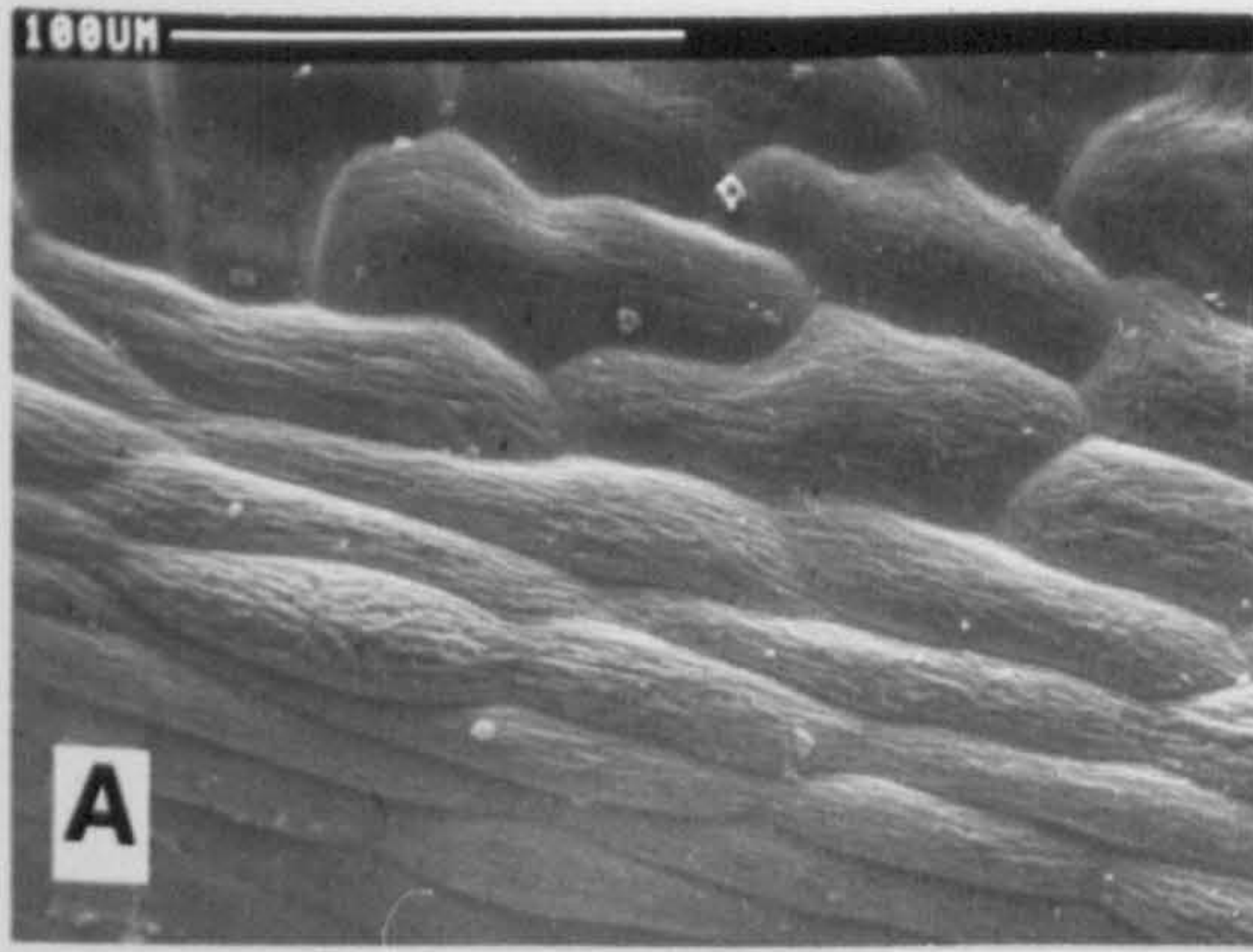


Plate 2. 2

A *F. multiflora* P162

B *F. cynanchoides*

C *F. cilinodis* P 148 X 300

D,E,F *F. cilinodis* P 148 trichome and epidermis

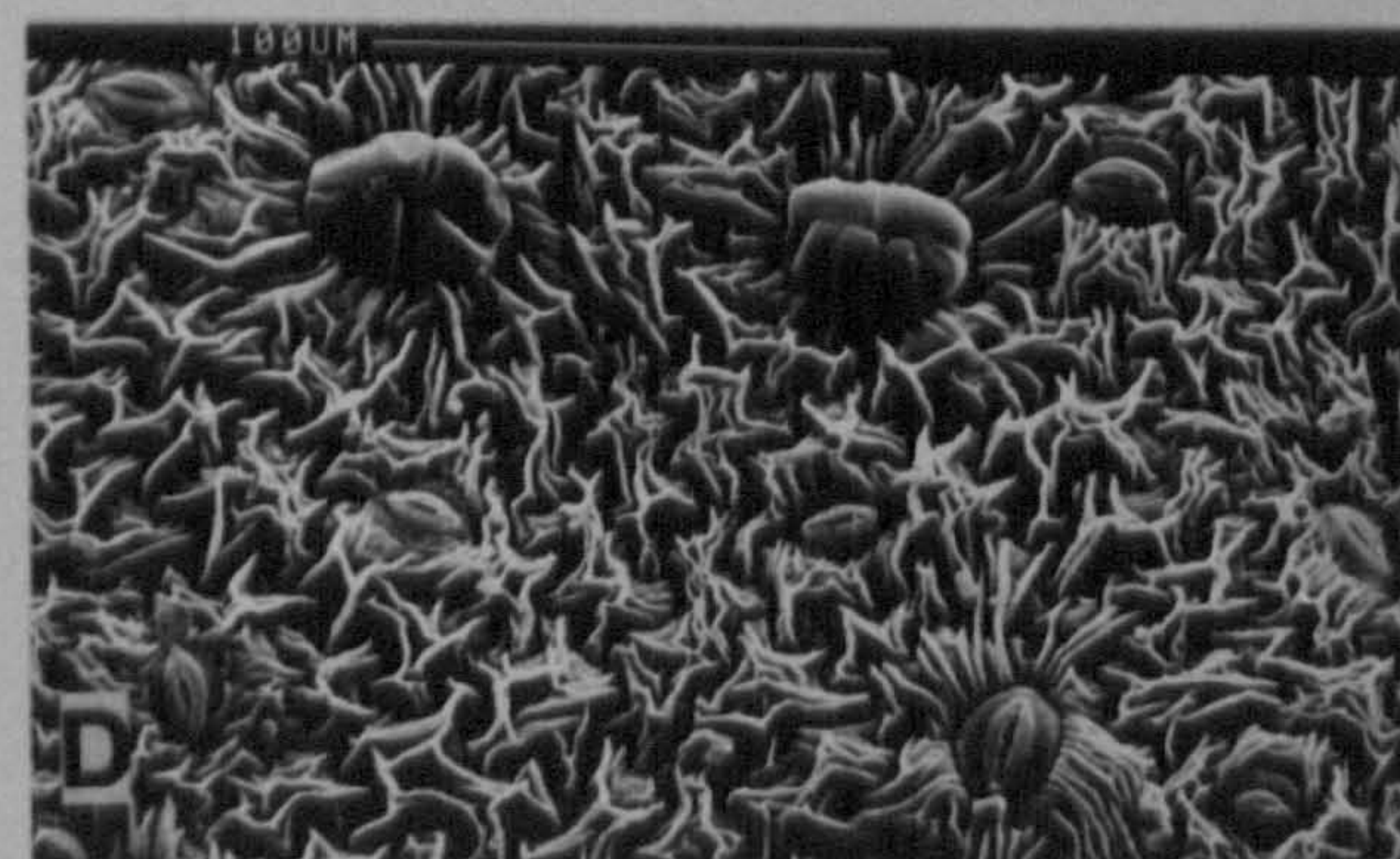
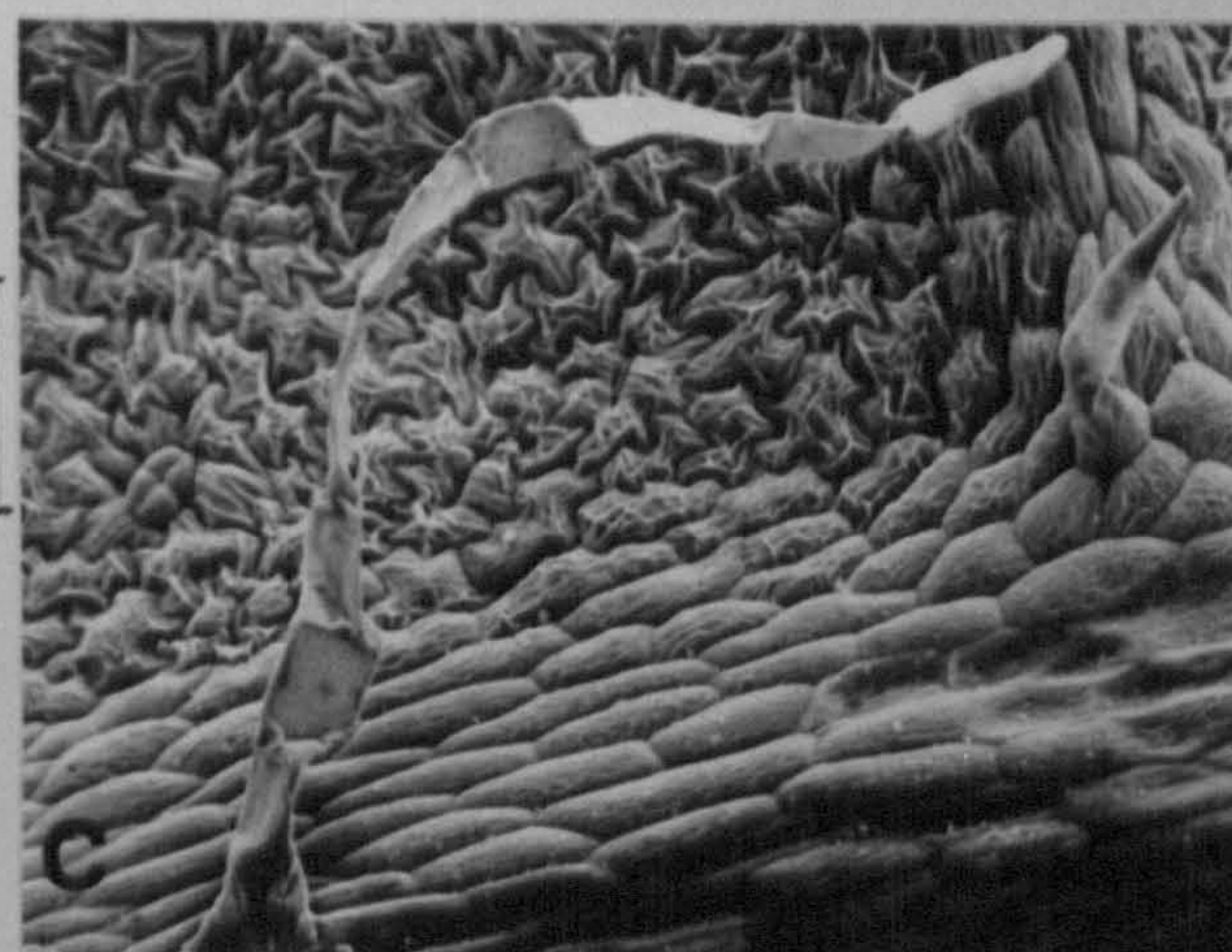
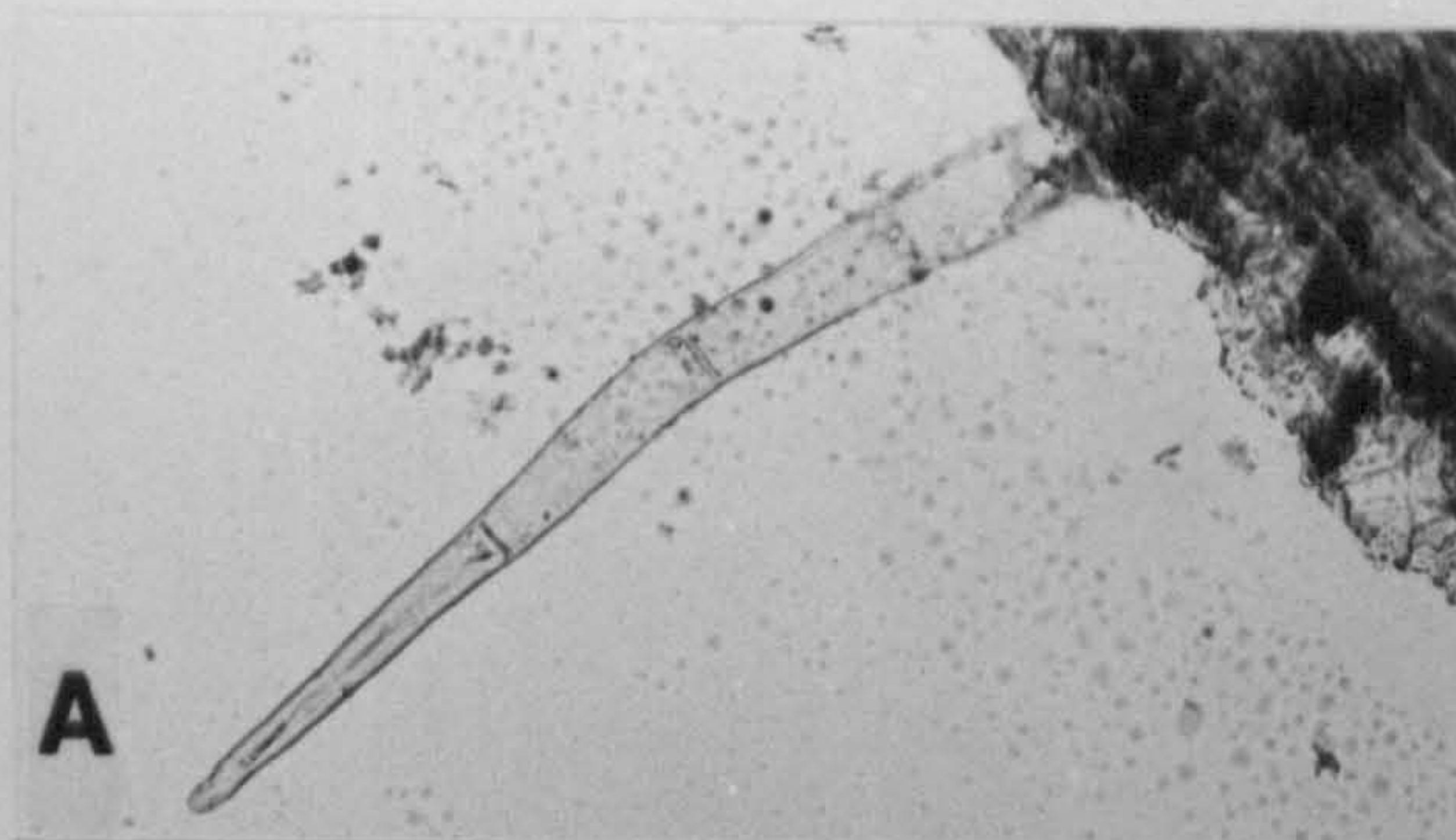


Plate 2.3 *R. sachalinensis*

A P57 3 celled hair

B P 115 14 celled hair

C P57 collapsed hair

D P 115 Tokyo note glandular trichomes

scale bar 100 microns

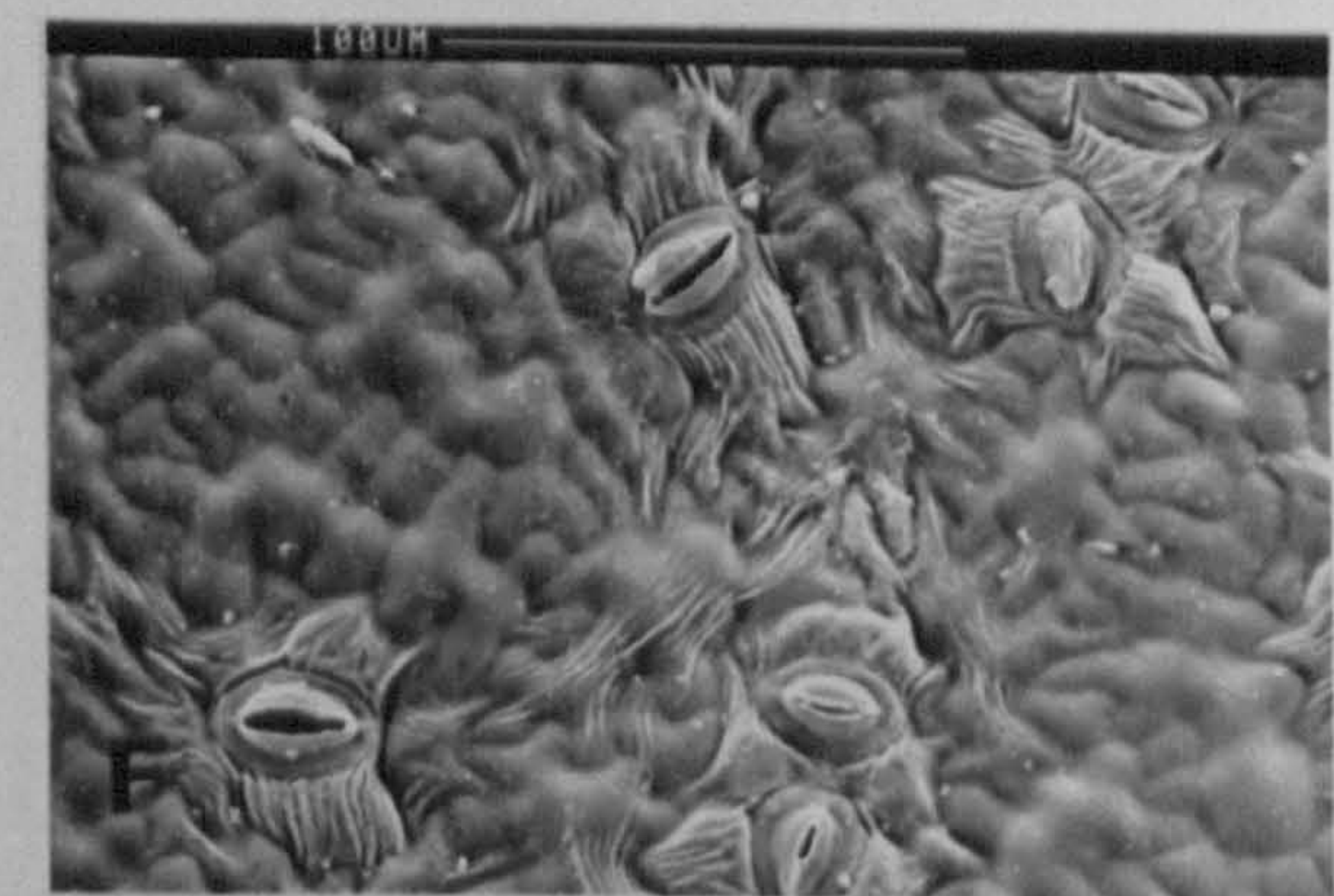
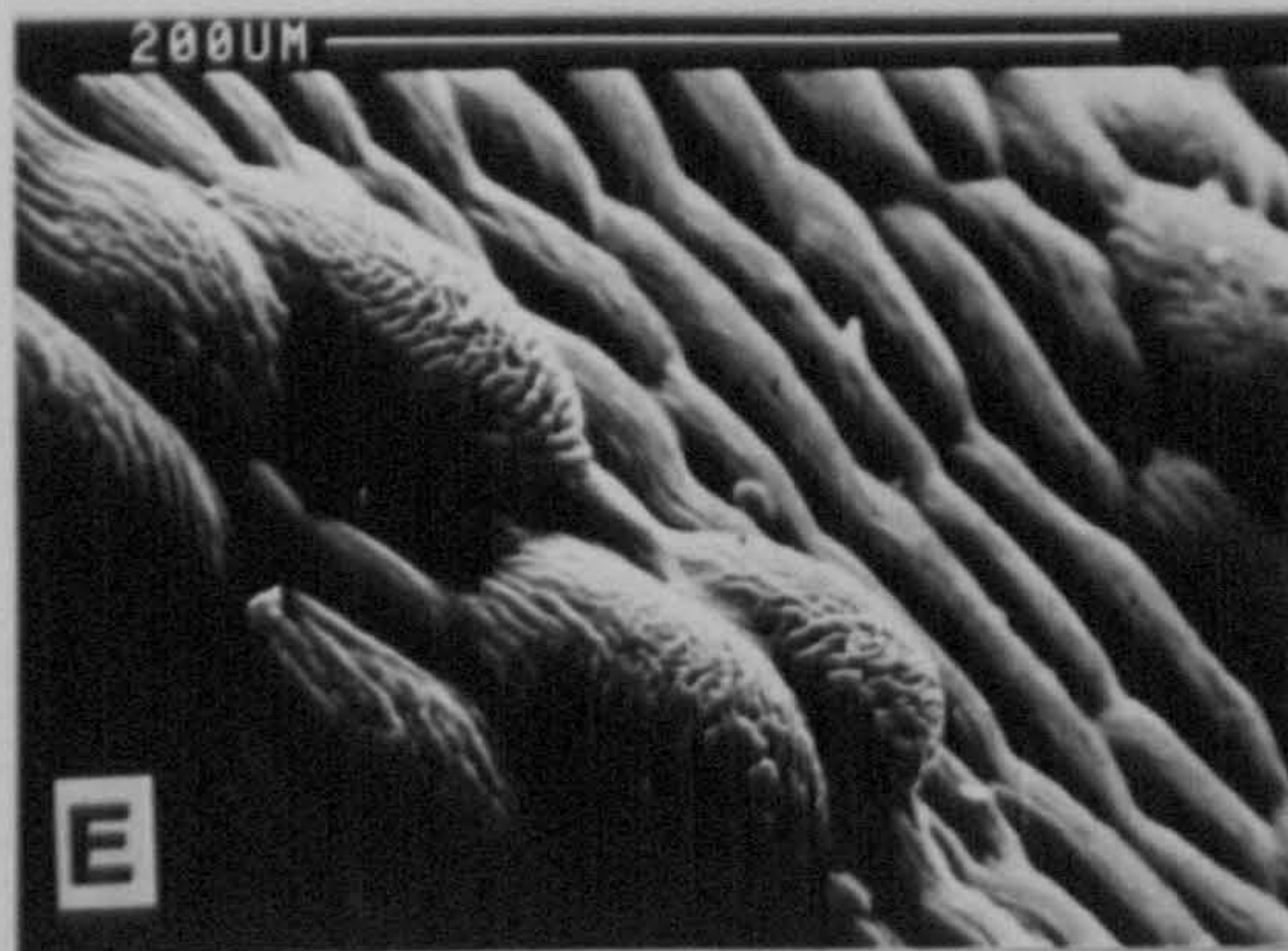
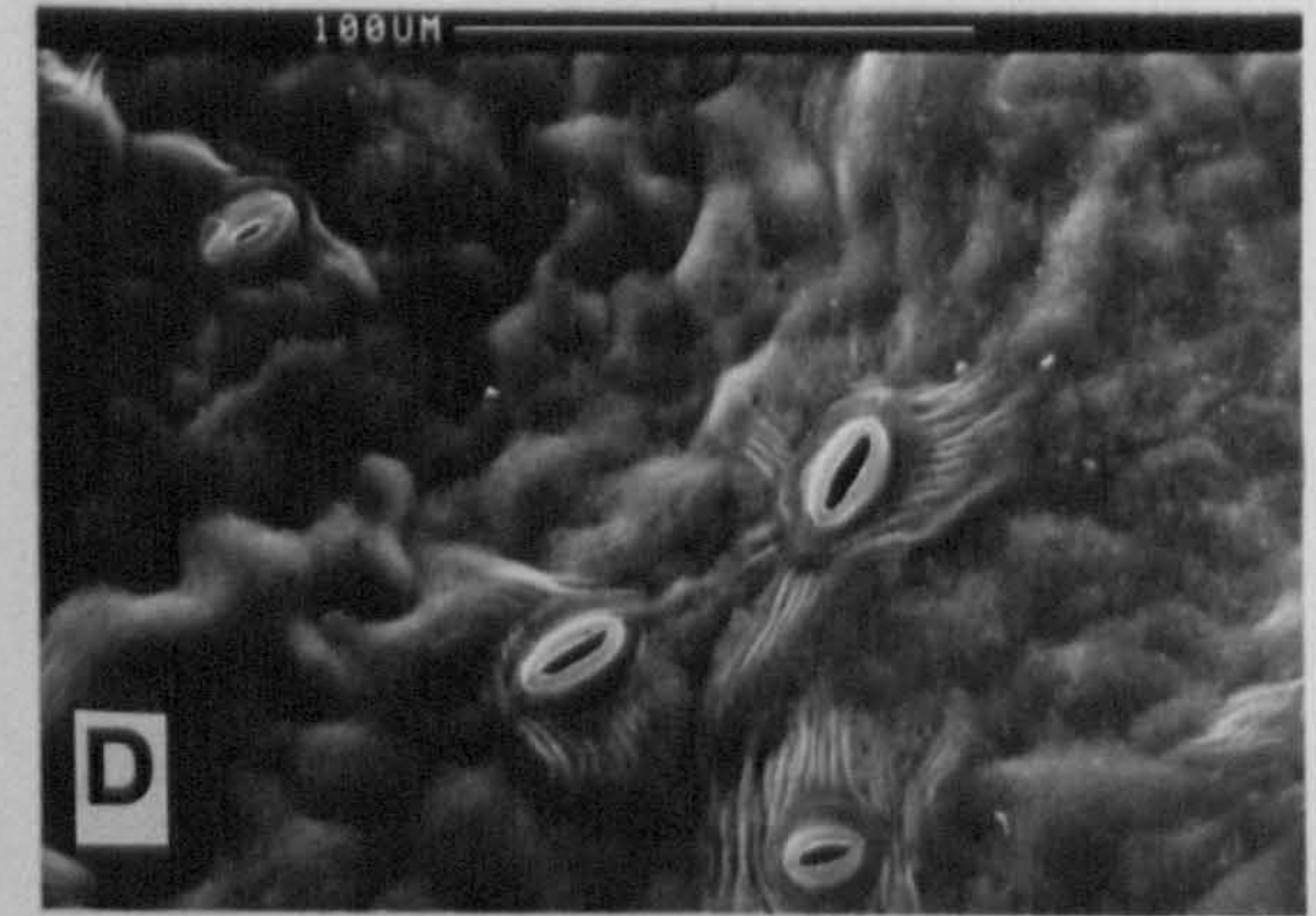
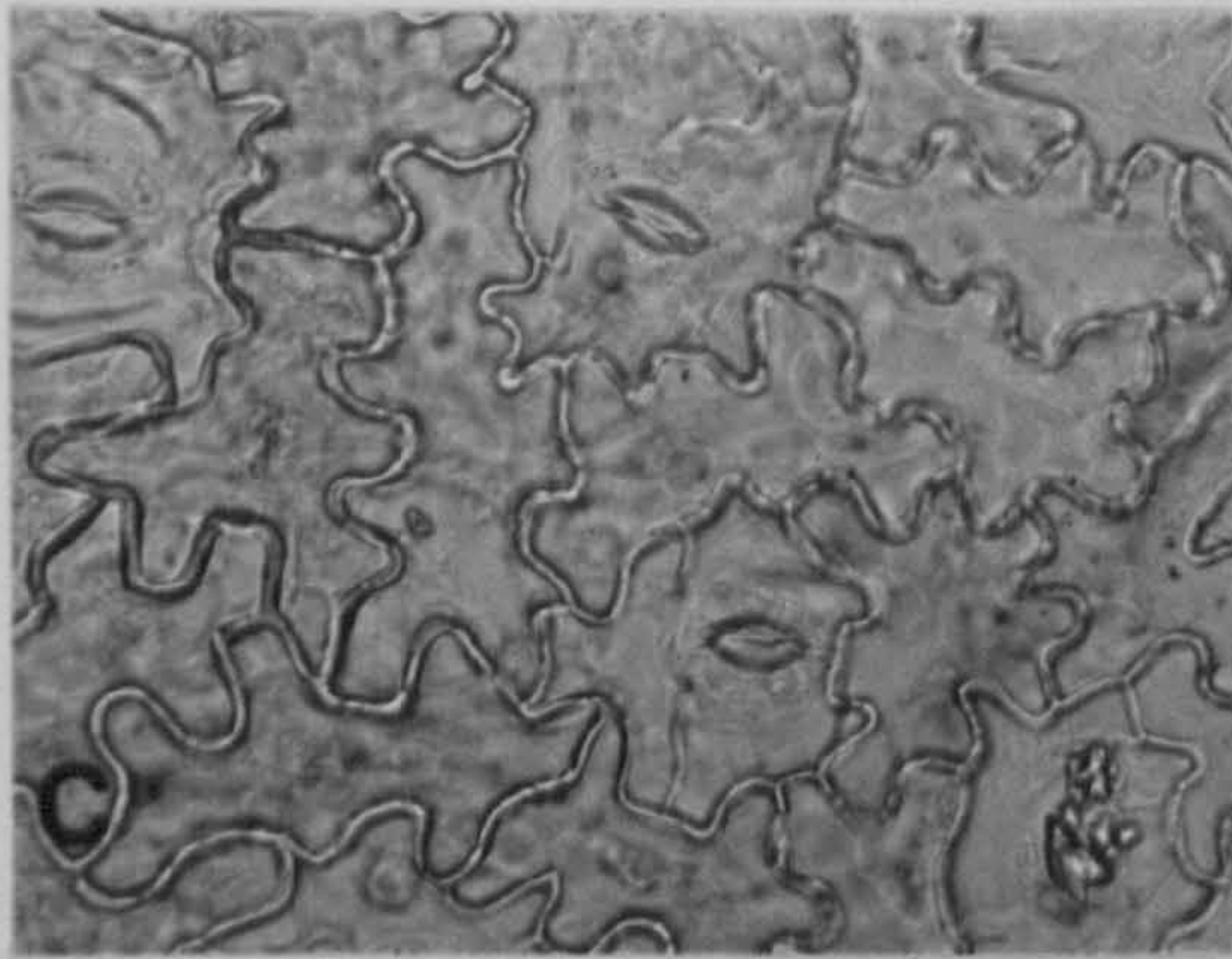
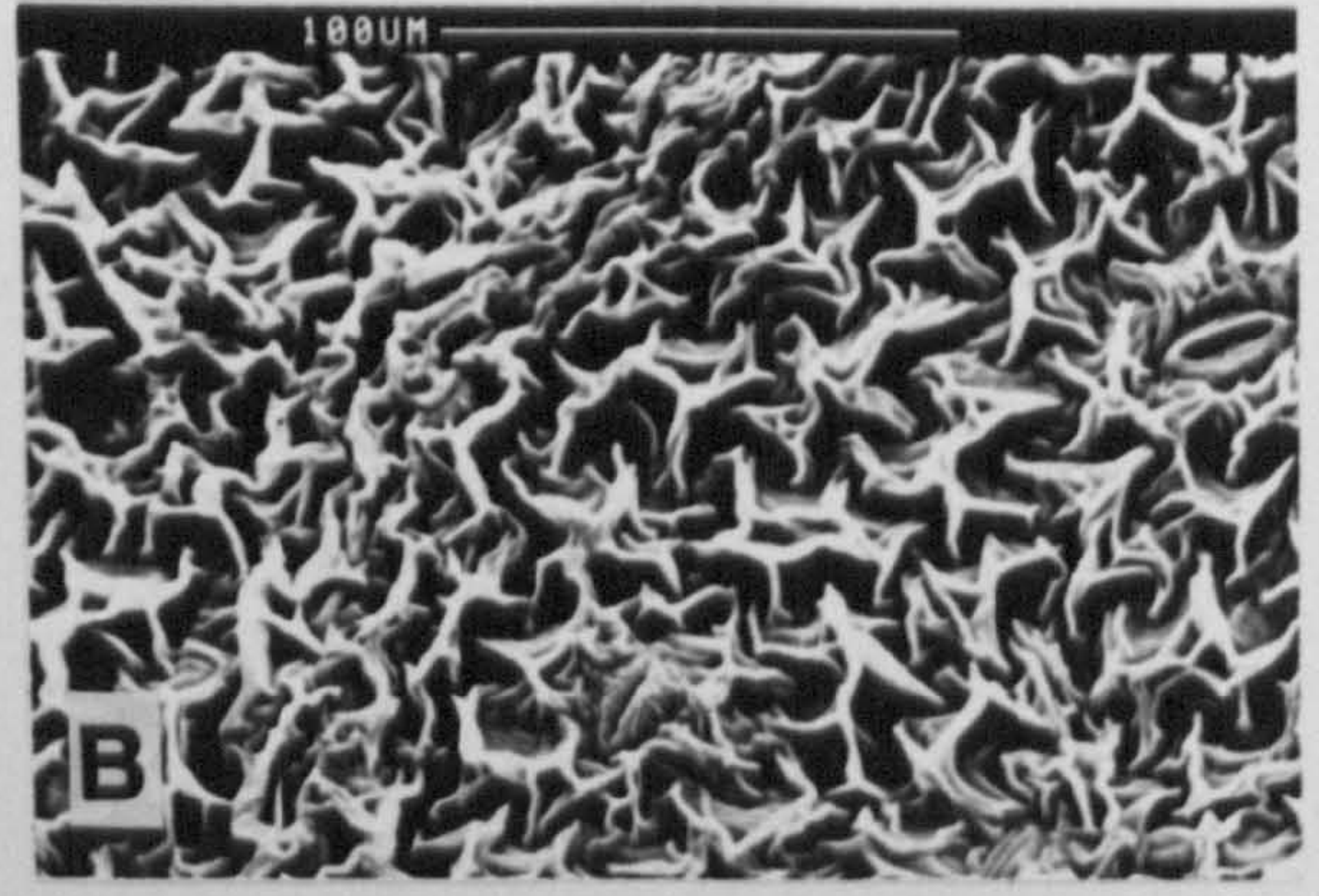


Plate 2.4

A *R. sachalinensis* P57

B *R. sachalinensis* Leningrad Type material

C, D, E *R. japonica* var. *compacta* P 2

F 4x *R. japonica* var. *japonica* P114 Tokyo

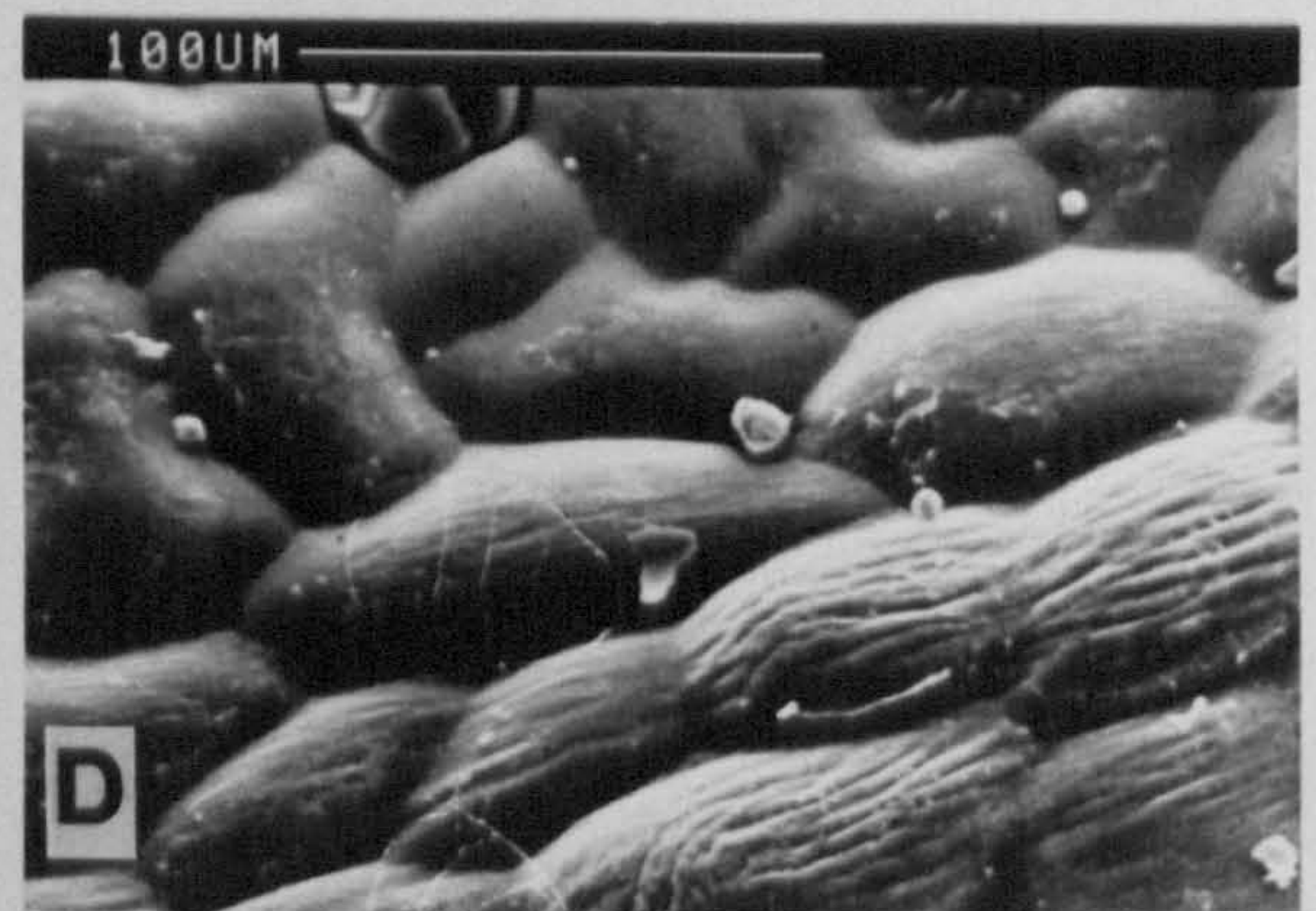
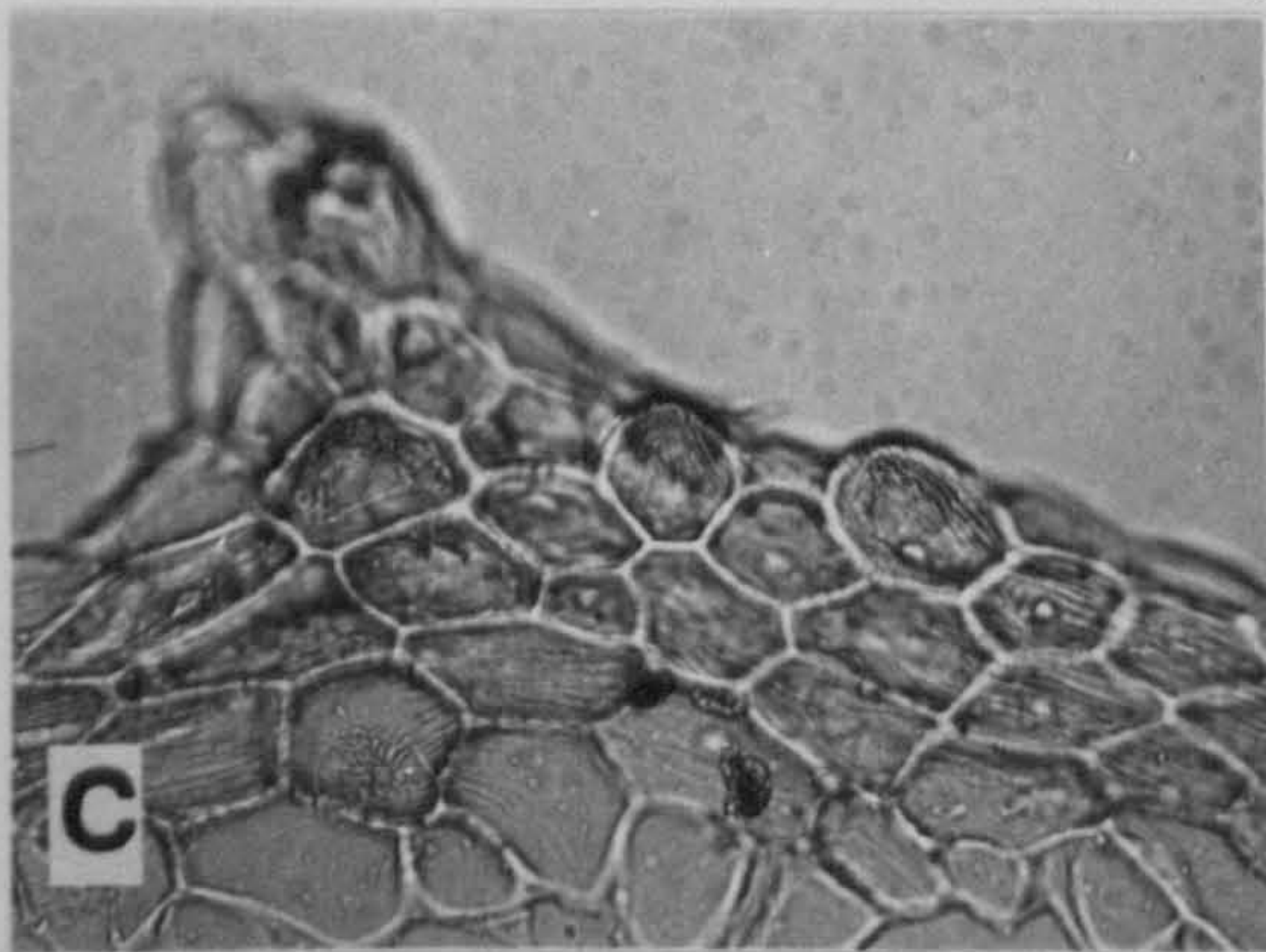
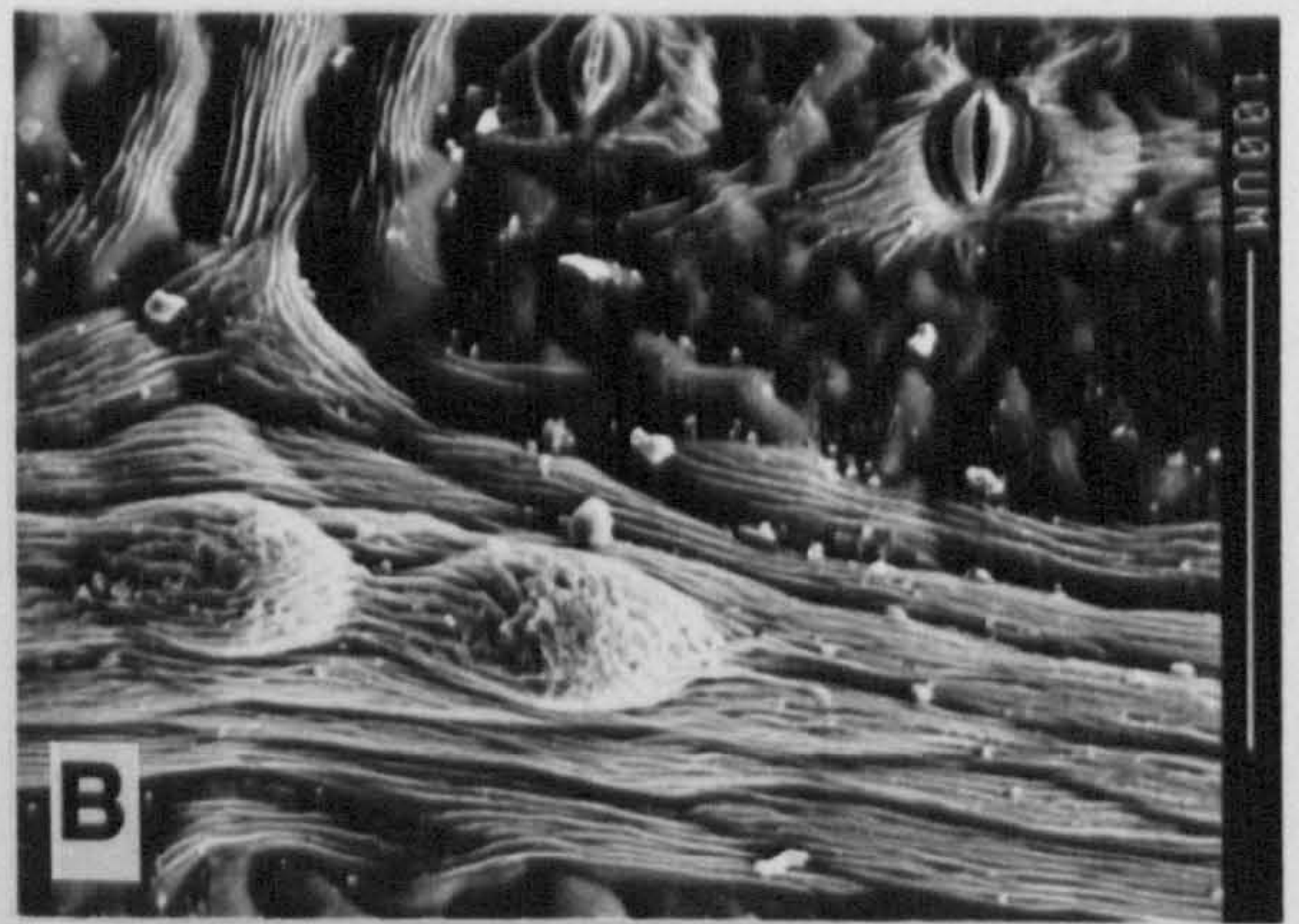
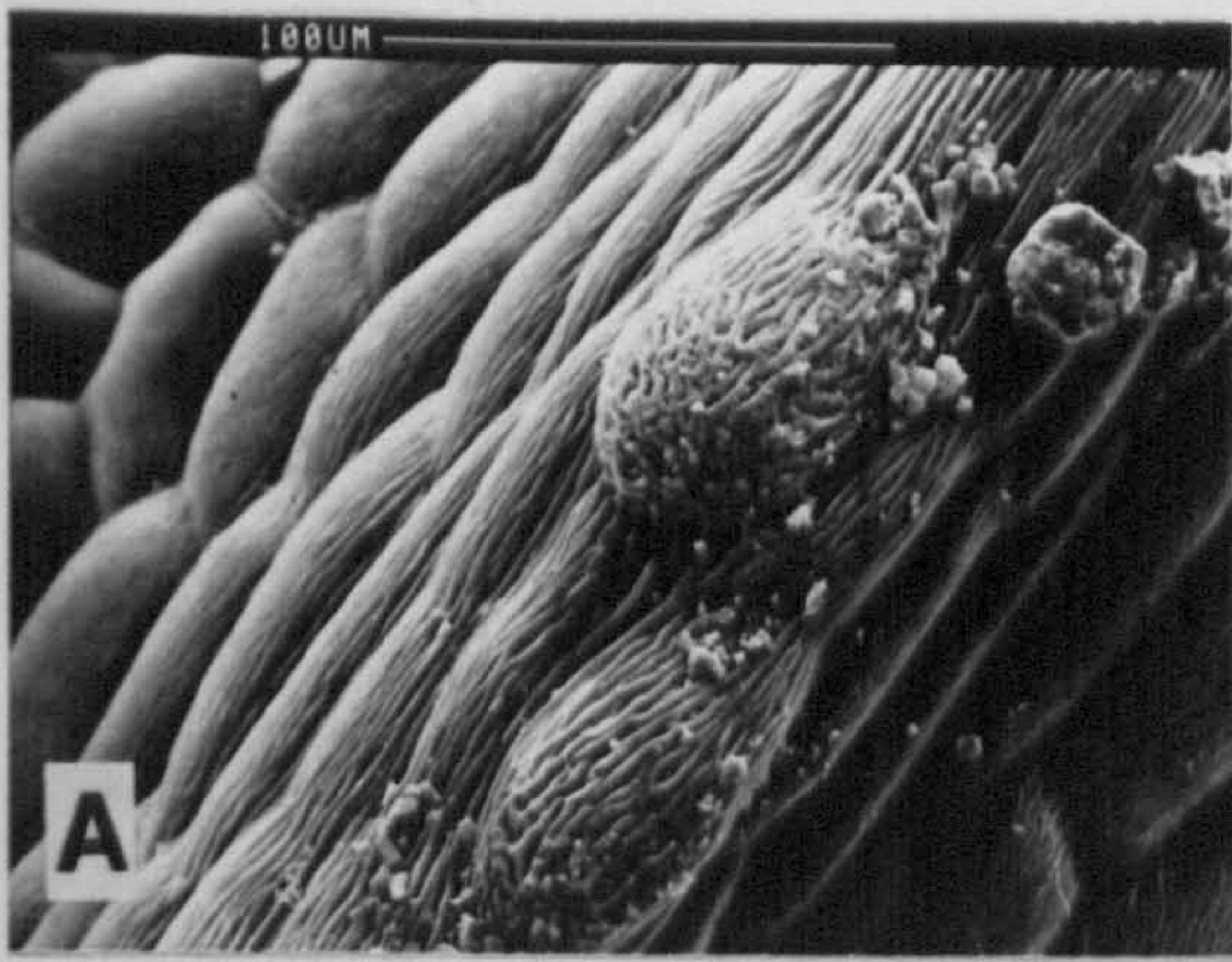


Plate 2.5 Japanese and Chinese *R. japonica* accessions

A Nasu mountains Japan P105a $2n = 66$

B Tokyo P114 $2n = 44$

C,D Peking P113 $2n = 88$

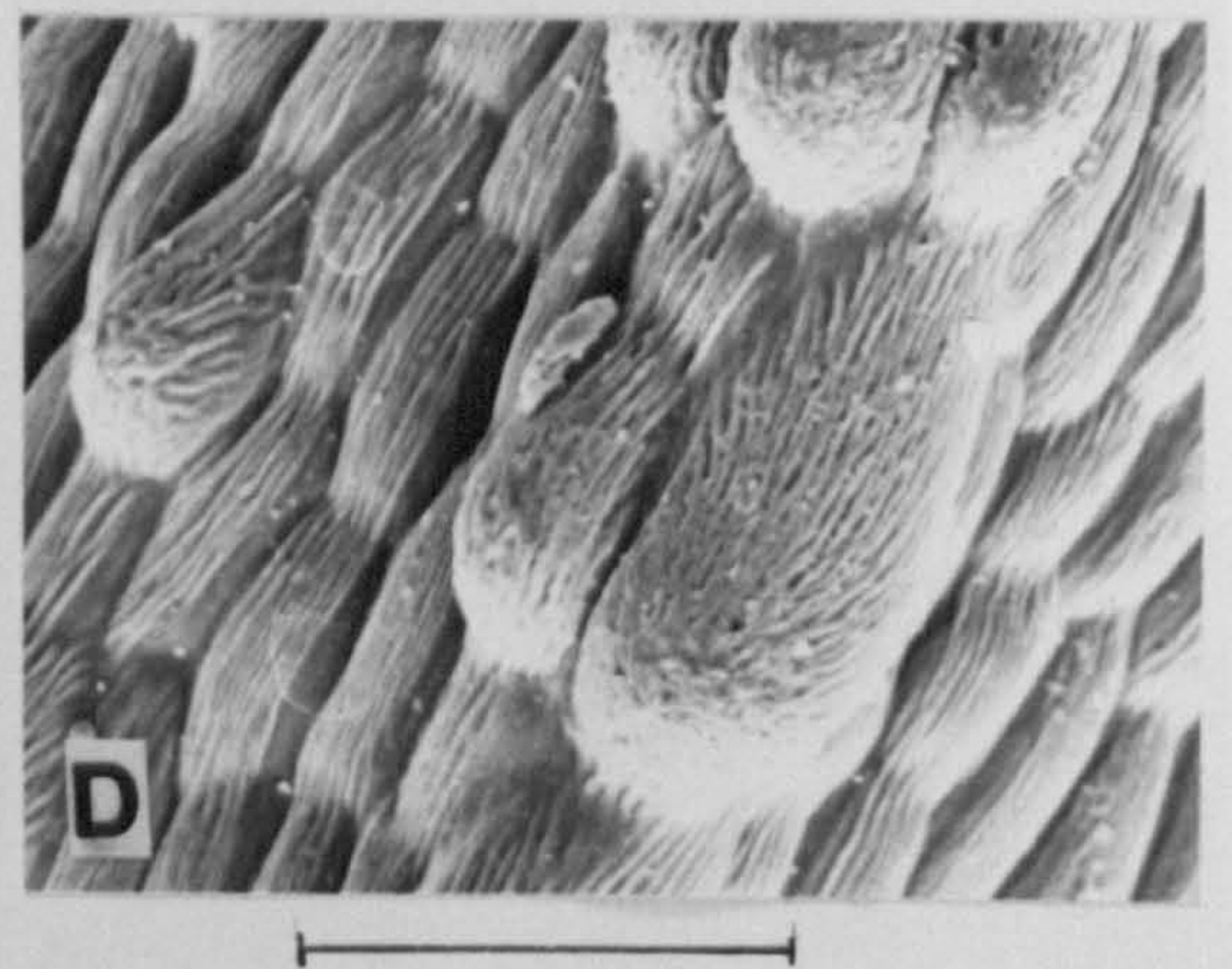
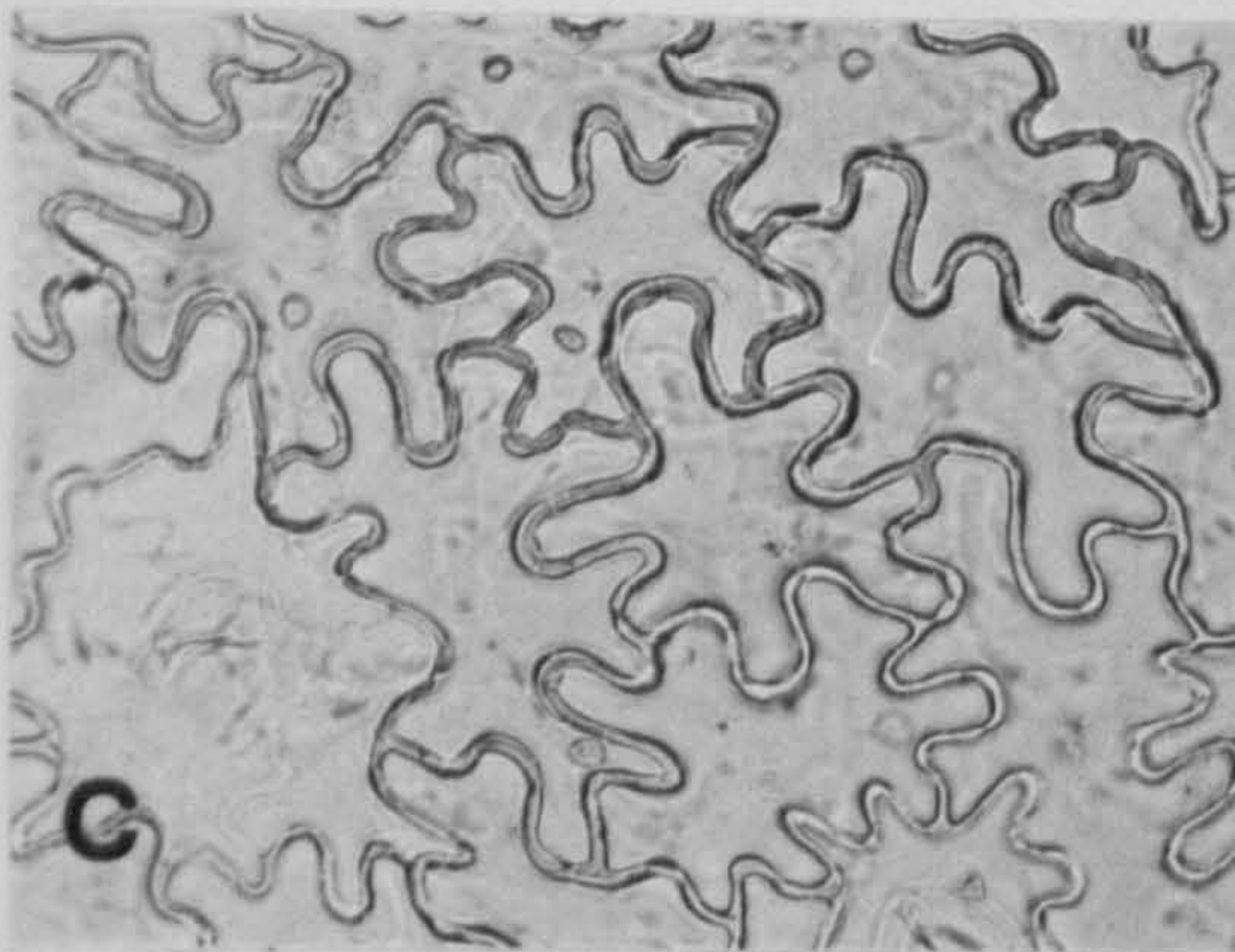
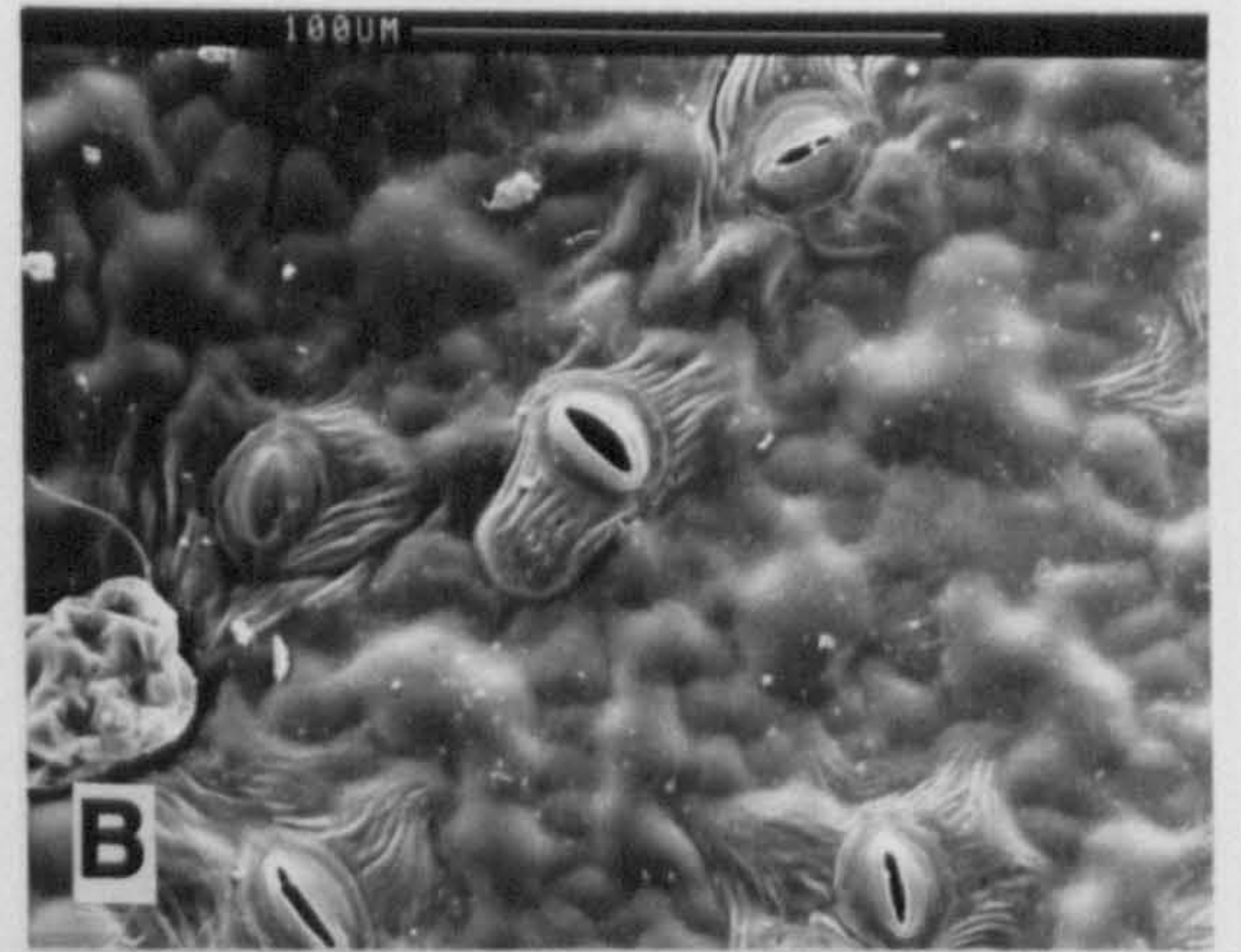
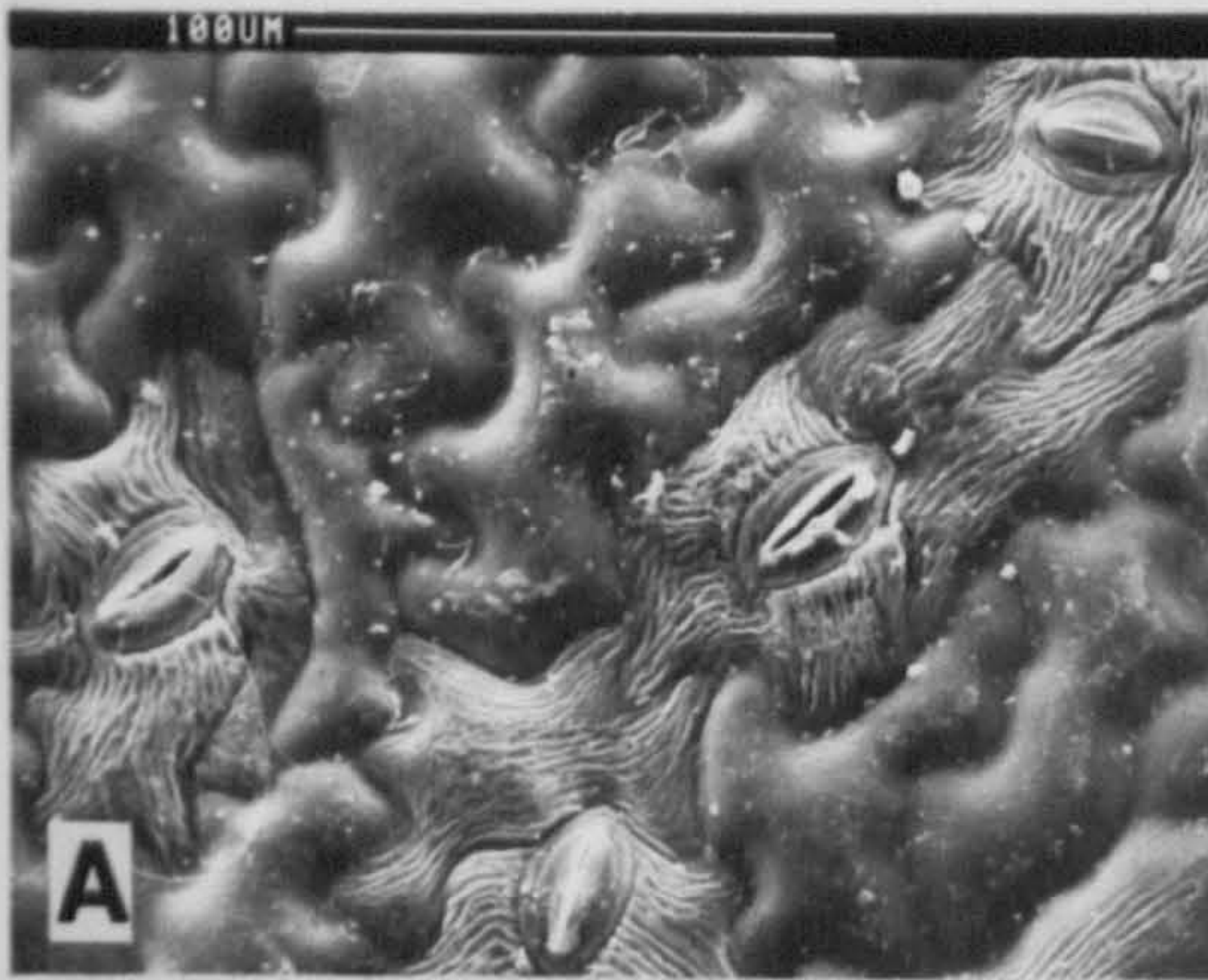


Plate 2.6 Octoploid *R. japonica*

A Peking P113

B P166

C P12

D P166 scale bar 100 microns

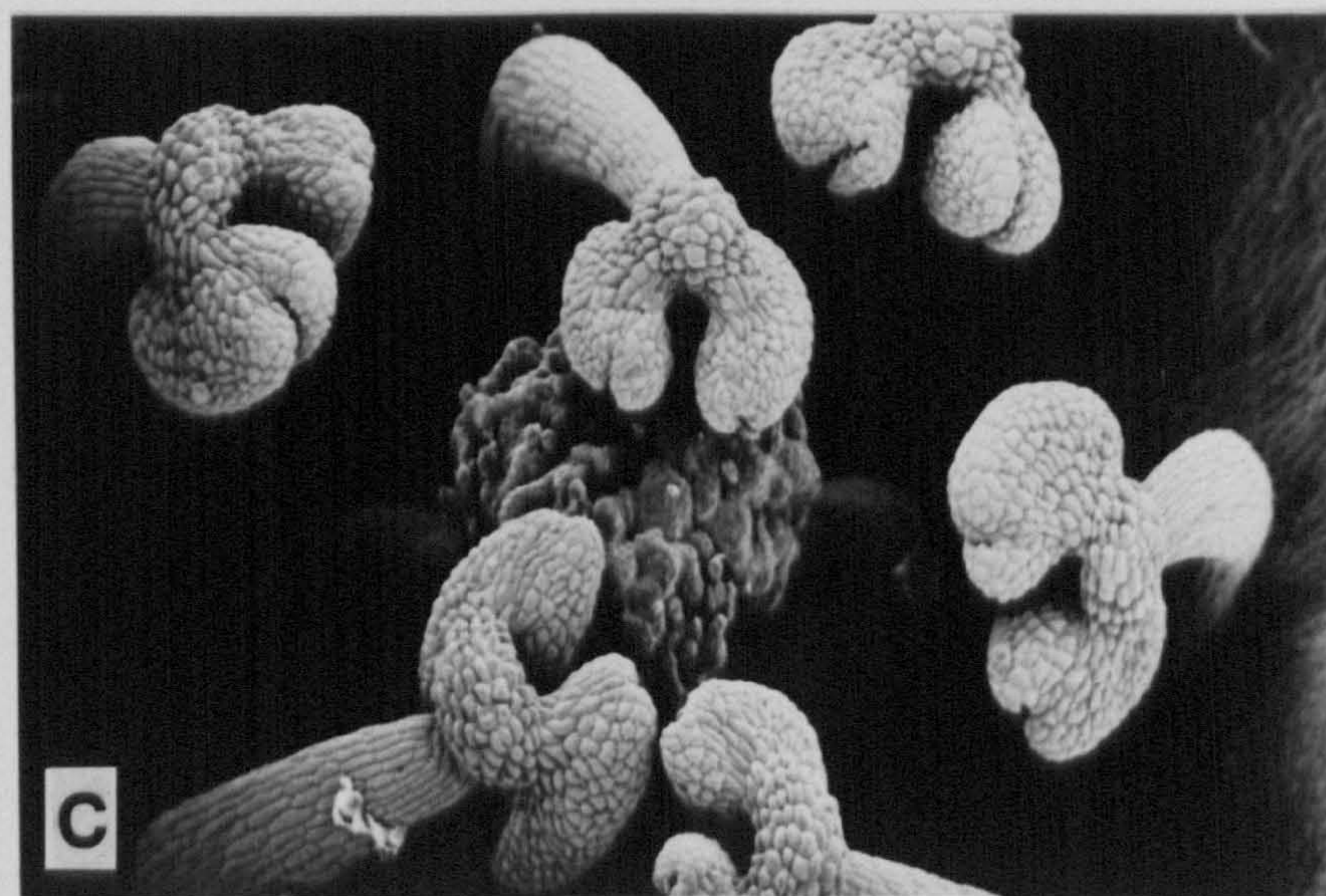
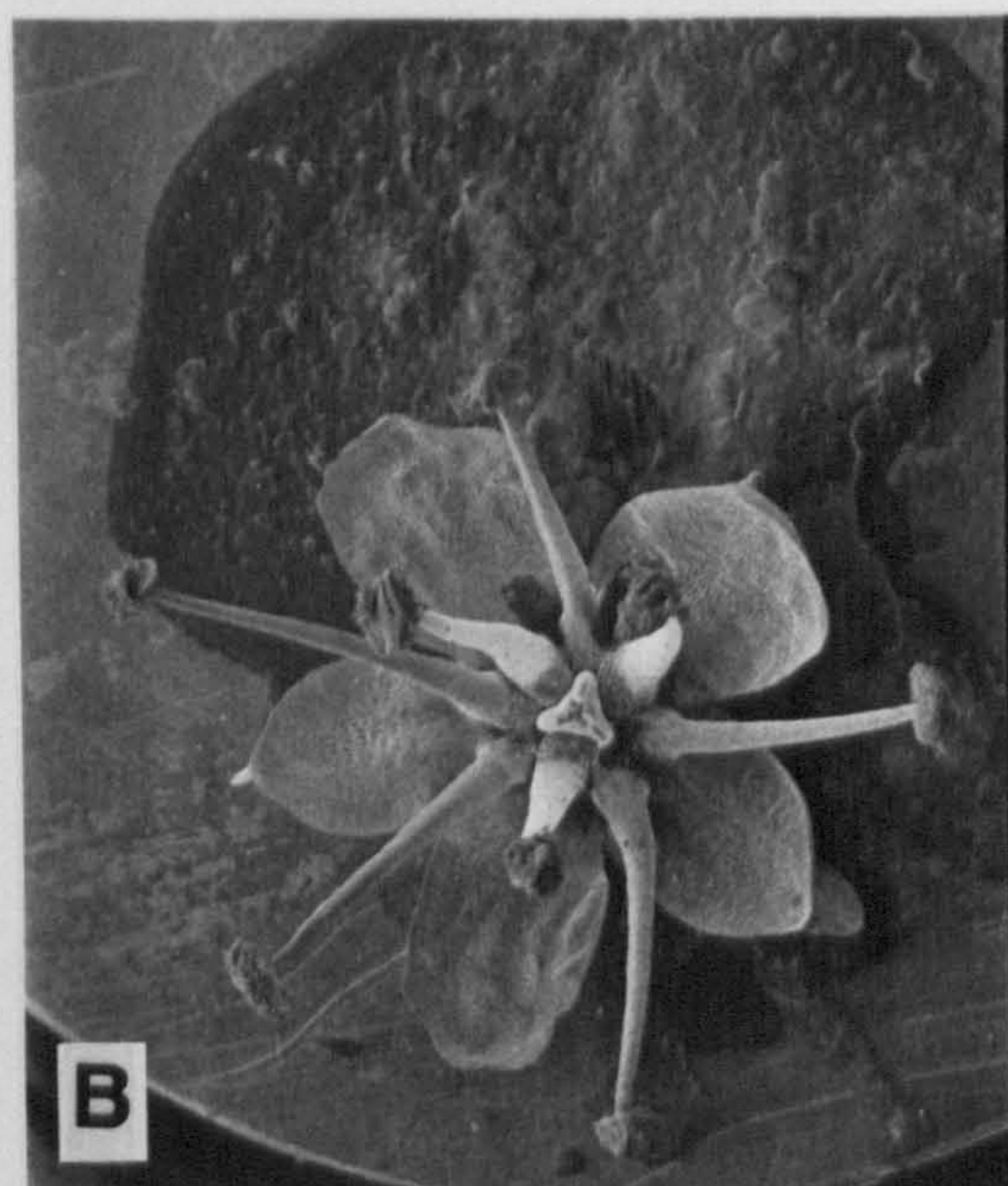
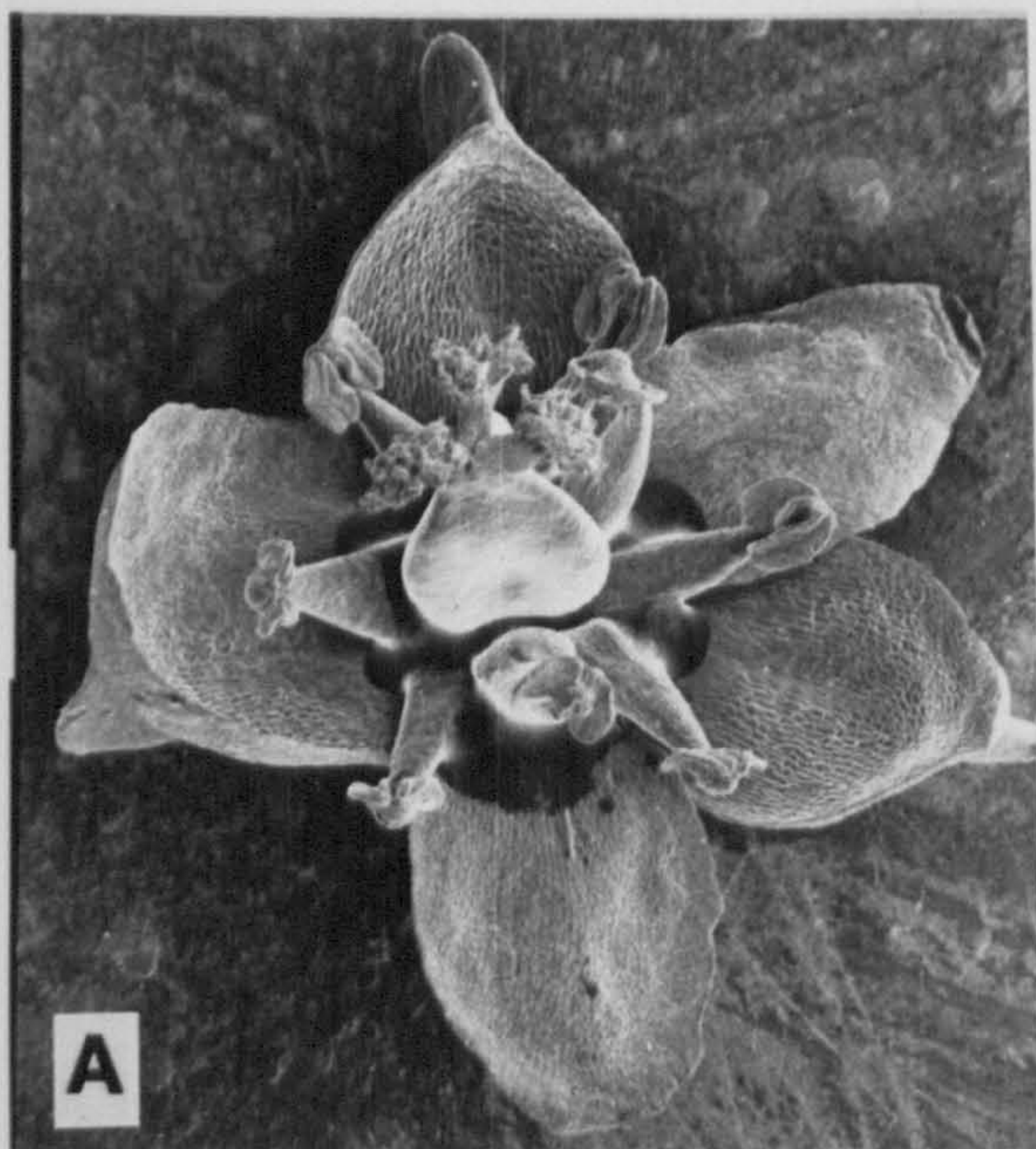


Plate 2.7 Floral morphology

- A** *R. sachalinensis* P57 male - sterile scale bar 588 microns
- B** Tetraploid *Reynoutria* P78a male - fertile scale bar 893 microns
- C** *F. convolvulus* X 75

Chapter 3

Cytology of the species

3.1 INTRODUCTION

3.1.1 CYTOLOGY

It can be said without fear of contradiction that plant cytology is still at the alpha stage. Stace (1980) noted that on a world level the most fundamental cytological information, that of chromosome number, is known only from 15-20% of angiosperms. Regarding the types of cytological data of use to taxonomists Bennett (1984) offers 16 different areas of measurement spread between the following major categories; cytological, genetical, molecular and cytochemical. This information ranges from chromosome number and ploidy level to DNA base sequences, though in the foreseeable future the latter (due to their expense) will have applications only with important crop species.

The ultimate aim of the cytotaxonist is an exploration of species interrelationships, resulting from a reconciliation of cytological data with morphological and other data. The type of cytological data usually used are chromosome number, base number, ploidy level, chromosome shape, karyotype symmetry and the longitudinal differentiation of chromosome segments as visualised by the various banding techniques. The meiotic behaviour of chromosomes in both species and hybrids, and the quantity of DNA per genome, are also of value.

The relationship between chromosomal and morphological evolution is an interesting one, and suggests that

morphological evolution and chromosomal evolution can occur independently of each other. In some instances chromosomal evolution outstrips morphological evolution and one has the situation where great variation is found in ploidy level, DNA amount per 2x genome and karyotype within what is generally recognised as a single taxonomic species, as in the case in Scilla autumnalis L. (Ainsworth et al 1983) and Crocus speciosus Bieberstein (Jones 1983). The other extreme is found in the Liliaceous genera of Aloe, Gasteria and Haworthia where several hundred species are all represented by the same singular bimodal karyotype. On a different level other puzzling karyotype changes may be found. In Allium (Jones and Rees 1968) and Festuca (Seal 1983) for example, related diploids and tetraploids may be found in which the diploid has more DNA than the tetraploid. Again, aneuploidy, a normally rare or deleterious phenomenon, has found favour in taxa such as Claytonia virginica L. with its remarkable aneuploid series from $2n=12$ to $2n=191$. Whilst the basic mechanics of karyotype change such as pericentric inversions, translocations, fusion and fission cycles, duplications, deletions, and (more recently) transposable elements (Jones 1983) are well known, the same cannot be said of the reasons for such extremes of karyotype conservatism and chromosomal anarchy.

One school of thought would say that the great diversity that exists in relation to karyotype symmetry, ploidy level, base number and DNA amount is not necessarily random, but is a consequence of different underlying strategies in which the various components, such as breeding system, degree of

DNA heterozygosity, and the various cytological gambits for increasing or reducing recombination, are ultimately responsible for determining the karyotype. K. Jones is one such person who seems to regard the karyotype as more than just the sum of its parts, and for whom the karyotype is seen as a unit with a function '... an arrangement of genetic and non-genetic chromatin advantageously disposed for the purpose of controlling recombination between chromosomes' (Jones 1984). Indeed the analysis presented by Stebbins (1971 p.88) suggests that there are certain constraints on the karyotype. By plotting the proportion of chromosomes with arm ratio of $<2:1$ against ratio between largest and smallest chromosomes in a complement he arrived at 12 different categories of karyotype symmetry. Significantly 2 of the 12 categories are unoccupied, which tends to suggest that not every imaginable permutation can exist, and 4 of the others are occupied by Crepis taxa. It may well turn out that some combinations of centromeric position and size bimodality may be 'forbidden' since they are particularly disadvantageous, or that others such as the Crepis types may only be of fleeting occurrence in evolutionary terms since the attendant disequilibrium might lead to increase in recombination and perhaps in a rapid burst of morphological evolution.

Brandham (1983) looked at possible mechanisms for maintaining the extraordinary bimodal Aloineae karyotype. Whilst he found that asymmetrical interchanges occurred spontaneously at a frequency of 2.4% in the Aloineae in nature these seemed to remain as heterozygotes.

Experimental crosses between two interchange heterozygotes gave the expected 1:2:1 Mendelian ratio between non interchange homozygote, interchange heterozygotes and interchange homozygote, which demonstrated that there was no selection against them at meiosis. He suggested that interchange homozygotes may be selected against in nature, and that the easiest route to interchange homozygosity (self fertilization) is denied them due to the self-incompatibility system found in most members of the Aloineae. This is an interesting demonstration of the effect of the breeding system on the evolution of the karyotype.

Bennett's (1984) work suggests that karyotypes are not randomly organised and 'that chromosomes may be ordered according to simple principles of widespread biological application and functional significance'. He proposed that individual chromosomes lie in a predictable sequence based on long arms being associated with long arms and short arms with short arms, and termed this the 'natural karyotype'. He further suggested that 'nature often appears to favour a karyotype which (for a given range of arm sizes) tends to maximize the differences between pairs of non-adjacent corresponding arms, while perhaps minimizing the differences within pairs of adjacent corresponding arms'. The restraints that the natural karyotype places on future chromosomal evolution might be a factor in why the relative shapes of chromosomes are maintained during evolution even though large changes in DNA c-value also occur.

Changes in karyotype symmetry in related species or genera are often assigned a polarity with respect to evolutionary change. The prevailing opinion is that symmetrical karyotypes are primitive and asymmetrical ones advanced. Jones (1983) regarded this as insufficiently supported dogma and in any case it is in direct contradiction of the views of zoologists who hold the opposite view and have ample evidence for it. I do not see why evolution has to be in the same direction for all plant taxa, and can well believe that symmetrical karyotypes are advanced in one genus and primitive in another.

3.1.2 BASE NUMBER AND PLOIDY LEVEL IN POLYGONUM L. S.LAT

The most commonly occurring base numbers within the Polygonaceae are 10 and 11, sometimes at high ploidy levels. The base number 7 is encountered amongst the numerically not very significant genera of Oxyria and Koenigia, whilst 8 characterises Fagopyrum and 9 Calligonum. Rumex provides the most diverse picture cytologically. Although existing predominantly with the base number 10, not only are there some very low chromosome base numbers, but these low base numbers are found in dioecious plants with sex chromosomes. Smith (1969), working with one of these (Rumex hastatulus Baldw.), found races with base numbers of 4 and 5 and various numbers of sex chromosomes. These species are, he suggested, derived via chromosome fusion from closely related perfect-flowered species with base numbers of 9 and 10.

By way of an introduction to the cytology of my group of plants, previously published counts were studied with a view to examining the distribution of base numbers and ploidy levels within Polygonum s.lat. Although this is a somewhat crude exercise, owing to the nature of the data and the assumptions that need to be made in a compilation of this type, it was nevertheless hoped that major trends would be highlighted as would certain spurious or erroneous reports, in a manner more useful than a bald statement of base numbers and ploidy levels recorded for a genus or section. Chromosome reports were taken from the various standard chromosome count compilations (Bolkhovskikh et al. 1969, Moore R.J. 1973,74,77, Moore D.M. 1982, Goldblatt 1981,84,85, Ping-Sheng 1985), notwithstanding the fact that synonymy, where it exists, results in counts being recorded for the taxon more than once, which can introduce a certain bias to the results. Further the reports may well be skewed in favour of diploids and low polyploids since they are more easily counted accurately than are high polyploids.

Secondly, the following conventions have been applied. Although in this family the diploid counts are easily recognised, counts which would otherwise imply a base number of more than 13 have been treated as polyploids, unless the base number or ploidy level would then be an odd number. Hence $2n=32$ is treated as a tetraploid with a base number 8, $2n=24$ is treated as a diploid with a base number 12, and $2n=34$ is treated as a diploid with a base number of 17. Approximate counts are disregarded for the purpose of base number computation unless the apparent base number in

question is corroborated by other counts within the section. Counts above $2n=90$ have been also disregarded for the base number compilation owing to the increased incidence of aneuploidy, combined with a certain lack of precision that can accompany such reports.

No record was made of the number of reports for a particular taxon, except in the case of section Tiniaria where obviously a more thorough study of the literature is required. The results of this compilation are shown in Fig. 3.10.1 It may be seen that 10, 11 and to a lesser extent 12 predominate. 8 occurs in three sections and is the only record for section Helxine, whilst 7 is the sole record for Koenigia and occurs nowhere else. It is interesting to note that the base number 9 does not occur in any of these sections, though it is the most frequent base number in the genus Calligonum. A notable absurdity thrown up by this exercise is the case of Polygonum chinense L. in section Persicaria. By application of the ground rules to its reports of 22, 26 and 32, diploids with base numbers of 11 and 13 and a tetraploid with base number 8 are produced; fortunately this sort of anomaly is very infrequent. It may also be seen that chromosome base number has scant respect for the taxonomic divisions of Polygonum s.lat., base numbers of both 10 and 11 occurring in 5 sections, and 10, 11 and 12 in two. This would tend to vitiate against those who would split section Tiniaria along chromosome base number lines. All groups except Koenigia and section Helxine are represented at the hexaploid and octoploid, albeit at fairly low frequencies, although section Polygonum

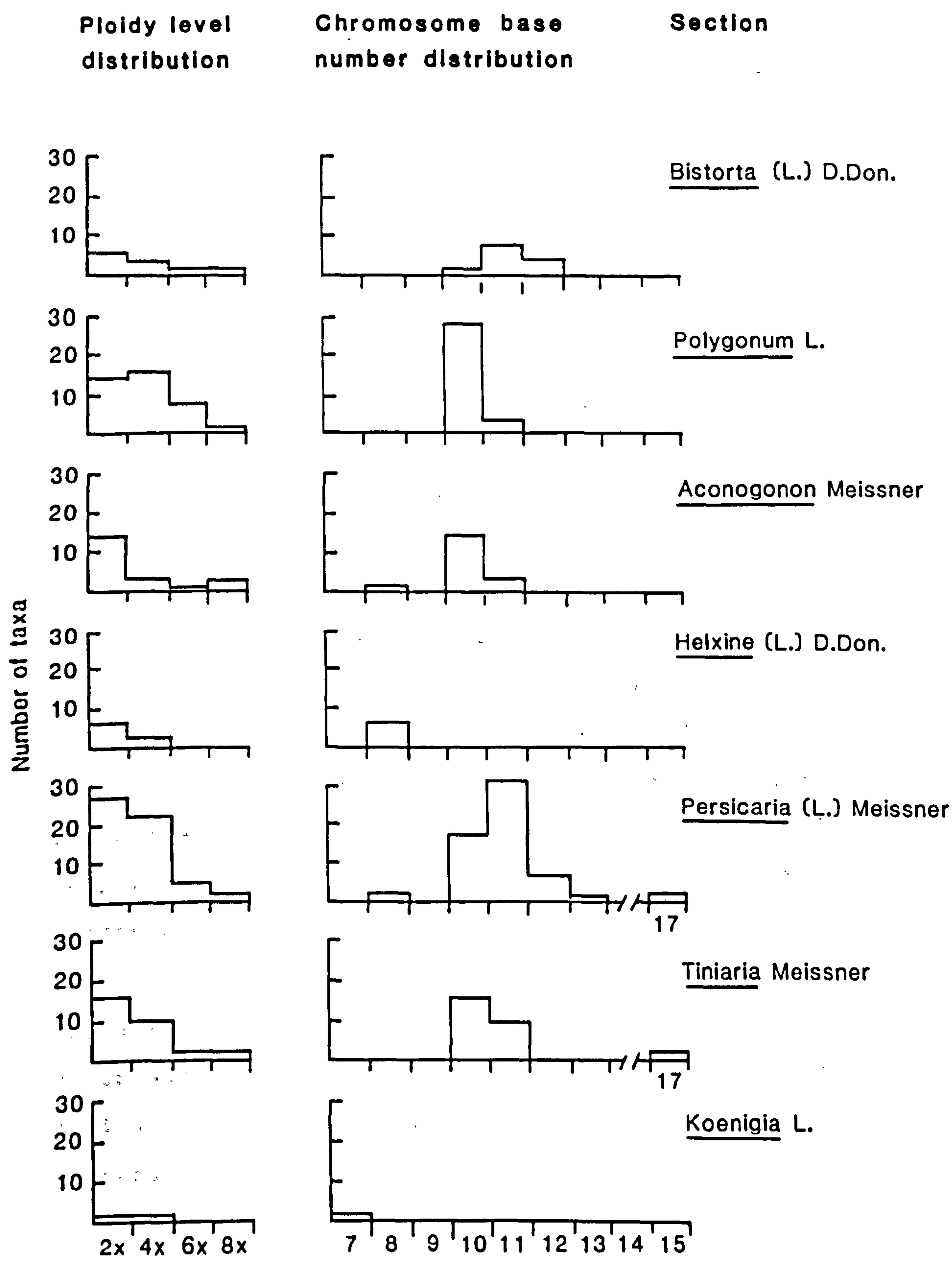


Figure 3.10.1 Distribution of ploidy level and chromosome base numbers in the different sections of *Polygonum* s.lat

has quite good representation at the tetraploid and hexaploid levels.

3.1.3 POLYPLOIDY

When an organism possesses 3 or more sets of the haploid genome (i.e. when $2n=3x$ or more) it is said to be a polyploid. Such situations are generally easy to spot, with one or two exceptions. In organisms with diffuse centromere agmatoploidy can occur, since chromosomes may break up without loss of centric activity. Thus a diploid with a complement of 12AL chromosomes may give rise by chromosome fission to an agmatoploid series with 24 BL half size, or 48 CL quarter size chromosomes. Since this same diploid may also give rise by chromosome doubling to a true polyploid series with 24 AL and 48 AL chromosomes, ploidy level determinations must be undertaken with care in such organisms. Another difficulty is found in those taxa in which the lowest haploid number for the genus or family is very high, say 20 or more. In such cases, although highly suggestive of polyploidy, one has no real way of knowing if they are diploids or polyploids with extinct diploid progenitors. Indeed Stebbins (1971) suggested that any genus with a base number higher than $x=12$ has probably been derived via polyploidy from groups having lower chromosome numbers.

The phenomenon of polyploidy is largely restricted to the plant kingdom; although there are examples from the animal kingdom, they are rather rare. However, the almost

universal separation of the sexes in the higher order of animals and the low incidence of parthenogenic taxa must place more barriers in the way of polyploidy as an evolutionary mechanism than is the case in plants. Some families such as the Poaceae have an extremely high incidence of polyploidy, more than 80% of species having undergone polyploidy at some stage of their evolution (Stebbins 1985).

The occurrence of polyploidy in many of the important crop plants, such as the allohexaploid wheat, and the discovery of methods of artificial chromosome doubling, led many to think that the increase in size and vigour of many polyploids when compared to their diploid relatives could be achieved by chromosome doubling. In other words, that it was polyploidy per se and not the genetic constitution of these polyploids that led to their increased performance. This has been shown not to be the case with autopolyploidy. Stebbins (1985), in a comparative planting experiment started in 1943 with the South African grass Ehrharta erecta Lam. and autopolyploid derivatives of it, found that not only did the diploids out perform the autopolloids, but that they also appeared to be developing different growth habits in different habitats, some of which were maintained under uniform growth conditions.

Polyploidy can arise via several routes, the simplest being by a somatic doubling of the chromosomes without cell division. This chromosome doubling probably takes place fairly regularly in plants, but only when this occurs in a

shoot or rhizome meristem is it that polyploid flowers can arise. Another route is via the production of unreduced gametes. Marks (1966) had diploid strains of Solanum chacoense Bitter that produced high frequencies of unreduced gametes. These plants had regular bivalent formation, but the later stages of meiosis were very irregular with failure of the second division. Polyploid seed could be produced by self fertilization in such a plant. A third related route is via the production of triploids resulting from a fertilization event in which one of the gametes was unreduced. Since triploid meiosis is notoriously irregular and restitution nuclei commonly formed, the fertilization of one of these by a normal haploid gamete from another plant would give rise to a tetraploid with a somewhat broader genetic base. In the case of chromosome doubling occurring in a hybrid F_1 individual that is sterile due to lack of genome homology the outcome is a fertile polyploid with a wide basis of genetic variation. One immediate consequence of polyploidy to a newly formed polyploid is that a different pattern of regulation of genetic heterozygosity is brought about by tetrasomic inheritance. Whilst in a diploid the hypothetically advantageous gene combination Aa is rapidly lost, since 50% of the offspring will have the homozygous parental types, in the polyploid equivalent $AAaa$ self fertilization will only lead to 5% of parental types, with all manner of combinations in between. The same is true where one considers the expression of potentially advantageous double recessive gene mutation products, the polyploid producing 2.5%, and the diploid 25% of parental types. In short, diploids can give rise to more rapid

expression and turnover of recessive gene combinations, whilst polyploid buffering ensures slow loss of heterozygosity combined with low expression of recessive gene combinations.

The question of the distributional and ecological correlates of polyploidy is not one that can be answered by simple generalizations. Early publications tended to conclude that polyploidy was commoner at more northerly latitudes and that polyploidy made plants better able to stand the cold. These data came mainly from Europe where, according to Stebbins (1971), the flora as a whole does show an increased percentage of polyploidy at more northerly latitudes. The fact that in the rocky refugia in the high Alps above the snow-line the percentage of polyploids is no higher than that of the flora in the foot-hills and plains below suggests that more than climate per se is involved. Cytological investigations of rain forest floras have, if one accepts high base numbers as evidence for ancient polyploidy, even higher percentages (85-95%) of polyploids (Stebbins 1971).

The belief now is that it is changes in local conditions that suit the evolution of polyploids e.g. catastrophic environmental changes caused by factors such as glaciation or mountain building, particularly if these come after a long period of stability. This would perhaps leave diploid taxa finely tuned genetically to the prevailing conditions, whilst the polyploids, perhaps already present in low numbers, would with their broader genetic base be to a

certain extent pre-adapted to the climatic changes. The changes in environment produced by an irregular and unpredictable glacial retreat might well produce an increased incidence of polyploidy in the migrating flora.

Stebbins (1985) gave an example of the incidence of polyploidy in related genera but with different habitat preferences in support of his secondary contact theory. This proposed that polyploidy is more likely to be successful in diploids that have been separated for some time and which subsequently come in contact again and hybridize. He suggested that this will be much more likely to occur in species with a 'patchy' distribution as opposed to those with a continuous distribution such as in a climax forest or riverine habits. In the related genera Populus and Salix, the former is mainly confined to river banks (continuous distribution) and is mainly diploid. Salix, however, has some riverine species and others that have a patchy distribution on mountain slopes and early stages of forest formation and among these there are many polyploids, some as high as $2n=190$.

There are some correlations between life-form and the occurrence of polyploidy. Annuals tend to exhibit the lowest frequency of polyploidy and temperate perennial herbs the highest, with the position of woody plants being intermediate. It must, however, be said that polyploidy is much more likely to survive in plants with effective vegetative reproduction particularly so if meiosis is irregular. Annuals on the other hand led a more precarious

existence, dependent for survival on good seed set, and polyploidy would mean bearing the added load of synthesising twice the amount of DNA of the diploid progenitors. One could envisage that the advantageous gene combination and hybrid vigour would have to be considerable in order that the new polyploid established itself in the first place.

Much has been written on the relative importance of autopolloidy and allopolloidy in the generation of successful polyploids. Autopolloids are theoretically much easier to generate since they can arise in a single step via somatic doubling of chromosome number. Allopolloids and the range of segmental allopolloids on the other hand must first hybridize (whether at the species or ecotype level) and then undergo chromosome doubling. Both must of course pass the barrier of meiosis if they are to become sexual polyploids. This is easiest for the strict allopolloid, since the two genome combined in the initial F_1 are assumed to have low homology so that there is little pairing at meiosis, hence doubling the chromosome number produces an exact homologue for each of the F_1 chromosomes, so that at a stroke fertility is restored. A newly formed autopolloid on the other hand has a different problem, since its chromosomes have too much homology resulting in a high frequency of multivalents at meiosis and consequent loss of fertility. Segmental allopolloids fit in between these two extremes. Autopolloids and some segmental allopolloids need to find another way past the meiotic barrier and they achieve this by the use of multivalent suppressor genes. The Ph gene in wheat is the best studied of these, and strains without this gene show a

large amount of homoeologous pairing, compared with the strictly bivalent formation in strains with it. The occurrence of Ph-like genes is thought to be involved in suppression of multivalent formation in some autopolyploids and also in amphiploids where the difference in genotypes is not reflected in pairing ability.

On the one hand Stebbins (1985) has taken the view that autopolyploidy (except where the parents of the plant that produces the polyploid were genetically heterozygous) is of relatively minor importance in evolution: '...without hybridization autopolyploids, even when highly fertile, are greatly inferior to their diploid progenitors under field conditions or at best can maintain themselves but lack aggressiveness and the ability to invade new habitats'.

On the other hand Jackson (1982) believes that autopolyploids (with the appropriate preadaptations at the diploid level) are a force in the evolution of polyploids when they have a Ph-like system of multivalent suppression. Jackson considered that Ph genes operated by affecting the attachment site of chromosomes to the nuclear membrane. He suggested that this attachment is crucial to the whole realm of homologous and homoeol^ogous pairing. The lack of pairing in an F_1 hybrid could be because the attachment sites for the two genomes were too far apart to allow pairing.

In true autopolyploids and normal diploids, homologues are equally close so that pairing obeys probability laws that make it possible to describe expected pairing events with

appropriate models and equations (Jackson 1982). If this is the way that Ph genes operate it has certain consequences for the taxonomic use of chromosome pairing in F_1 hybrids, since the operation of one or a few genes could eliminate pairing in what in DNA sequence terms are homologous chromosomes (or vice versa). Another point is just how homologous in sequence terms do two chromosomes need to be in order to pair? The reconstruction of zygotene and pachytene nuclei in hybrids between Lolium temulentum L. and L. perenne L. by Jenkins (1985) has brought to light some very interesting information. Whilst there exists a difference of 50% (evenly distributed across the karyotype) in the amount of DNA, pairing was apparently normal with chiasma frequency comparable with that of the parents. Synaptonemal complex (SC) formation was complete in 40% of the bivalents. This accommodation of differences of 50% in DNA between chromosomes must indicate widespread non-homologous pairing as do the various fold-back pairings seen also with good SC formation. From this it is apparent that a lot of what is taken as homology in terms of synapsis is not necessarily related to homology in terms of DNA sequences. The role of SC's in chiasmata formation is not yet understood though crossing-over cannot take place without them. According to Jackson (1984) less than 1% of the DNA in a chromosome is bound by lateral components of the SC and thus available for crossing over. Hence 'only a very small part of the total potential homology can be viewed in any one cell, and it is likely that the same bivalent may have different segments in the synaptonemal complexes in different meiocytes' (Jackson 1984).

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The role of polyploidy in evolution may be considered at two levels: the evolution of new species, and the broader evolutionary significance. In other words, does polyploidy lead or follow when considering the evolution of higher taxonomic categories? At the specific level the production of new allopolyploid species such as Primula kewensis Hort. and Senecio cambrensis Rosser represent instances of one type of speciation, indeed possibly the only type of speciation observable during the life span of a taxonomist! But with such speciation we are not considering the evolution of novel characteristics merely the rearrangement or averaging out of pre-existing variation. The simple genetical facts of disomic versus tetrasomic (or even hexasomic!) inheritance require that the evolution of novel characteristics by means of gene mutation and gene recombination are accomplished more effectively at the diploid level. Conversely, such variation as is generated, unless immediately conferring some advantage to the diploid, can just as easily be lost again. Polyploids may be seen as storehouses of genetic variation produced by these diploids combined with repositories of surplus expendable DNA open to the accumulation of mutations. This is not to say that polyploidy is always ultimately an evolutionary dead-end. Work by De Wet (1971) and Marks (1966) indicates that gene flow between diploids and tetraploids can indeed take place. In Solanum chacoense, a self incompatible diploid hermaphrodite, certain lines produce unreduced gametes which circumvent the incompatibility system so that tetraploids can be formed (Marks 1966). Once established these tetraploids can still maintain gene flow with their diploid

progenitors, their pollen grains occasionally fertilizing the unreduced gametes of the diploid. Marks found a very low incidence of triploids in such crosses, and suggests that this system is capable of generating allotetraploids directly. De Wet (1971) listed various incidences of fertile dihaploids being derived from tetraploids, and in particular the case of a recently derived dihaploid from the agamospecies Dicanthium aristatum (Poir) C.E. Hubbard, that is rapidly replacing the parental tetraploid. He even went as far as to suggest that in some cases, where tetraploids and closely related diploids exist, that these diploids could have evolved via haploidy after the original diploid progenitors became extinct. Haploidy provides the added benefits of creating diploids anew even after the basic diploids became extinct. New bursts of evolutionary activity become possible at the diploid level, and gene exchange between ploidy levels increases variability also at the tetraploid level.

3.1.4 CHROMOSOME BANDING

There has been a long felt-need for techniques capable of positively identifying individual chromosomes within a complement but, until the advent of banding techniques, cytologists have had to rely mainly on chromosomes with satellites and secondary constrictions or some other easily recognisable feature, or on the cold-induced differential staining. The longitudinal differentiation of a chromosome may allow such identification to be made, and additionally can provide more information of organisation at the gene or

molecular level allowing the detection of such mutations as symmetric and paracentric inversions, thus providing new dimensions of variation and polymorphism to be investigated. A good example of the use of polymorphisms revealed by banding techniques is provided by Fukuda (1984). An examination of banding patterns in chromosome A of Trillium ovatum Pursh revealed that populations on the Pacific coast of North America exhibited considerable banding homogeneity, in contrast with the considerable variation in banding pattern found in specimens from the Rocky Mountain regions. Moreover, this variation correlated well with morphological variation, which it was suggested reflected the recent evolutionary history of the species, the Pacific coast having historically been a stable habitat, compared to the glacial advances and retreats that occurred in the Rockies.

Early work on the longitudinal differentiation of chromosomes was carried out by Darlington and LaCour (1940) on Trillium. He found that subjecting plants to low temperatures for up to two days revealed certain thinner, less densely stained areas in the chromosomes. These areas corresponded to regions which were characteristically overstained at interphase. Darlington (1965) considered this to be due to the non-replication of DNA in those segments concerned, and it has been subsequently demonstrated (Lima de Faria 1983) that heterochromation is replicated later than euchromation. Darlington also found that chromosomes of 'starved' cells characteristically contained 10-20% less DNA than expected. Darlington (1965)

defined heterochromatin as 'those parts of the chromosome which have the inherent property of failing to maintain the maximum nucleic acid cycle at mitosis...'. These allocyclic or cold-induced regions were found to be mainly terminal or situated at secondary constrictions. Although these techniques enabled researchers to do some quite sophisticated work on translocations and population polymorphisms (eg. Haga and Kurabayashi 1954), these were generally restricted to a few genera in the Liliaceae.

Modern banding techniques date back to Caspersson et al. (1968) and their discovery of banding produced by the fluorescent agent Quinacrine mustard. The great expansion in chromosome banding was initiated by the work of Pardue and Gall (1970) who in the course of in-situ hybridisation of a mouse satellite DNA sequence noted that the distribution of silver grains corresponded with the darkly stained areas at the centromeres. Because of this association these they were termed C (centromeric) bands. They subsequently became associated with the highly repeated constitutive heterochromatin. According to Macgregor and Varley (1983) C-banding stains predominantly satellite DNA regions, though there are exceptions such as the sex chromosomes of the chinese hamster where the C-bands contain little satellite DNA. (This is satellite DNA in the molecular biological sense, meaning that portion separated from the main pellet in ultracentrifugation).

The other major non-fluorescent banding technique is that of G-banding, this mainly differs from C-banding (which also

uses Giemsa stain) in that the alkaline hydrolysis stage of C-banding is replaced by a mild protease digestion, or an incubation in saline solution. There exist in the earlier literature certain inconsistencies of terminology in that banding patterns that would now be termed C-bands were then termed G-bands. Greilhuber (1977) made the following distinctions between C- and G-bands in plant chromosomes. Whilst C-bands represent constitutive heterochromatin, which is unconvertable chromatin that remains condensed as chromocentres during interphase, G-banding reveals '...chromosome segmentation in contracted dividing chromosomes along their whole length and consequently also in other regions than those occupied by constitutive heterochromatin'. Plant banding techniques according to Greilhuber (1977) reveal only C-bands, and he is of the opinion that plant chromosomes cannot physically have G-bands because they are more tightly condensed than animal ones. He produced some interesting data which explains why, in the case of Homo sapiens, the chromosomes are large compared with the DNA C-value. This is apparently because (pro)metaphase human chromosomes have $0.02\text{pg DNA } \mu\text{m}^{-1}$ chromatid length against 0.15 for Secale cereale and 0.33 for Ornithogalum virens, which are thus 7.5 and 16.5 times more condensed at metaphase than human chromosomes. Drewry (1982), however, has demonstrated some convincing G-bands in Pinus resinosa which, although some $20 \mu\text{m}$ long, are still 7 times more tightly condensed than human chromosomes. It is indeed regrettable that G-bands are not widely found in plant chromosomes, since it is G-bands rather than C-bands that have enabled such precise identification of the human

karyotype to be made.

In-situ hybridisation (Hutchinson et al. 1981) offers tremendous potential for cytologists in the future, since not only does it provide a means of identifying individual chromosomes by the pattern of silver grain deposition, but it also reveals the actual position of the gene loci on the chromosomes. At present though, certain methodological considerations limit its usefulness to the general cytologist, not least of these being the molecular biological expertise, equipment and back-up required. Even given these there are still problems with the inordinately long incubation times required with low level probes, or the high background encountered with the use of a 'hotter' radiolabel. Further, it is not yet discriminating enough to pick up low copy-number sequences, i.e. those genes which so interest the molecular biologists and plant breeders. The use of biotin-labelled probes gives more immediate results and, although systems for amplifying the signal are being developed for low copy number sequences, it will be some time before such techniques are widely and successfully practised with plant material.

The third major method of chromosome banding is that of fluorescent banding. In terms of level of discrimination it is midway between the detection of homologous DNA sequences of in situ hybridization on the one hand and the identification of heterochromatin on the other, since it can identify chromosome segments that are comprised exclusively or predominantly of a particular DNA base pair. Since 1968

a number of important agents have become available for chromosome fluorescent banding. First are the synthetic drugs such as DAPI (4,6-diamidino-2-phenyl indole), ethidium bromide and the related Hoechst 33258. DAPI and Hoechst 33258 are A-T specific and their mode of action is thought to be by binding in the minor groove of the DNA helix (Gale et al. 1981), whilst ethidium actually intercalates with the DNA helix. The second group of fluorescent agents are various naturally occurring antibiotics which inhibit the host's nucleic acid synthesis by binding to their DNA in various ways. Actinomycin's mode of action is by intercalation, whilst the precise attachment mechanism of chromomycin and mithromycin is not known. The base specificity and mode of action of these agents is shown in Table 3.0 reproduced from Schweizer (1980). Since poor contrast is obtained if these agents are used singly, they are generally used in pairs of complementary base specificity. A common combination is chromomycin (G-C specific) and the non-fluorescent methyl green (A-T specific). The use of two complementary fluorochromes with different excitation wavelengths allows simultaneous observation of a single cell with either stain, the other operating as the counterstain. A chromosome segment stained with a particular fluorochrome will show negative, positive or enhanced fluorescence, depending on the fluorochrome used and the relative proportion of the two base pairs present. For example using the G-C specific chromomycin, normal euchromatin will show positive fluorescence, regions that are exclusively G-C enhanced fluorescence, and regions of A-T negative or quenched fluorescence. In suitable material it is also possible to

Fluorochrome	Binding and/or fluorescent specificity	Contrasting stain	Binding specif.	Enhancement mechanism ^d	DNA in regions resistant to fluorochrome quenching
DAPI ^c	A-T	actinomycin D • (AMD)	G-C	e.t.	enriched in A-T base pair clusters ^b
Hoechst 33258	A-T	"	"	e.t.	
Quinacrine	A-T	"	"	e.t.	
DAPI	A-T	echinomycin	G-C and sequence spec.	b.e. ?	A-T rich; deficient of spec. G-C rich sequences ?
DAPI	A-T	distamycin A (DA)	A-T	b.e. ?	A-T rich, 5-methyleytosine ?
Chromomycin (CMA)	G-C	methylgreen	A-T	e.t.	enriched in G-C base pair clusters ^b
"	"	distamycin A	A-T	b.e.	
Olivomycin	G-C	netropsin	A-T	b.e.	

TABLE 3.0 BASE SPECIFICITY OF COMMONLY USED FLUORESCENT STAINS AND MECHANISMS OF ENHANCEMENT

Taken from Schweizer 1980.

identify two intermediate conditions indicating that a particular base pair is predominant.

3.1.5 CHROMOSOME BANDING IN THE POLYGONACEAE

In terms of published reports, dicots are under-represented compared with monocots with reference to C-banding. Mizianty (1984,1985), in his bibliography of banding references, listed 133 monocot but only 68 dicot references. This is attributable to the greater importance and research effort that cereal crops attract, and the fact that some large chromosomed Liliaceous species are particularly amenable to these techniques. However, species in genera of little economic importance and with small numerous chromosomes have so far received little attention. Only one species in the Polygonaceae is reported as having been banded (Rumex acetosa), Vana 1972 and that not in connection with karyotype elucidation but on account of its heterochromatic sex chromosomes.

Sex chromosomes, in contrast to the position in the animal world, are extremely rare in plants. The Polygonaceae, however, contain several examples. Sex chromosomes are atypical in a number of ways, one of these being the frequent occurrence in them of large blocks of heterochromatin. In R. acetosa the male is the heterogametic sex with $12AXY_1Y_2$, the female possessing $12AXX$. Whilst it is useful to have a related taxon to use as a positive control in banding studies, it must be borne in mind that sex chromosomes are a special case.

3.2 MATERIALS AND METHODS

3.2.1 MITOTIC PREPARATIONS

- 1) Actively growing root-tips were collected between 10.00 and 11.30, and pretreated for 24 hours in a refrigerator (4-6°C) in 0.002M 8 hydroxyquinoline.
- 2) The pretreatment solution was then replaced with 3:1 ethyl alcohol:glacial acetic acid fixative.
- 3) Roots were then stored in fixative in the refrigerator until required (not normally for more than a few weeks).
- 4) The fixative was removed and hydrolysis carried out with 5N HCl at room temperature for between 8 and 10 minutes, depending on the thickness of the roots.
- 5) The HCl was removed and replaced by 70% alcohol.
- 6) Meristematic regions were dissected out in a drop of 45% acetic acid with fine tungsten needles and transferred to a drop of 2% aceto-orcein on a cleaned glass slide.
- 7) The Meristems were carefully macerated, and a cleaned cover slip placed on top. The preparation was then carefully tapped out to separate the cells, and gently squashed on a pad of filter paper.
- 8) Slides were then gently heated over a spirit burner and examined under low power. Suitable preparations were sealed with rubber solution and examined using oil immersion lenses.
- 9) Photographs were taken using a Zeiss large universal microscope fitted with an M35 camera. Photographs were originally taken on Ilford Pan F film, but this was subsequently changed to the ultra fine grain Kodak

Technical Pan film and developed with HC110 diluted 1 in 60.

Karyotyping

Photographs of suitable mitotic preparations with a final magnification of x3000 were produced. Individual chromosomes were then cut out with fine scissors and matched by eye into pairs in diploid taxa or the appropriate multiple in polyploids. Arm lengths were then determined using a Leitz x8 magnifier with a built-in 1cm scale. Rearrangements were then made in order to get the best accommodation between visual matching, arm ratio and chromosome length. In order to produce karyograms the measurements for a particular chromosome were first averaged, and then, in order to compensate for differential contraction between cells, expressed as a percentage of the diploid, or 2x equivalent karyotype length.

Where possible DNA-corrected karyograms were also produced (see Figure. 3.10). In this method the basic unit was the chromosome number divided by half the ploidy level, and the karyograms were based on this. Microdensitometric and karyological data were combined by according a nominal value of 100% to the taxon with the highest 2C value (F. cilinodis), and reducing the other karyograms in length proportionally according to their 2C value. This does, of course, assume the even distribution of DNA along the chromosomes of all taxa, which should be the case in taxa such as these which appear to lack significant quantities of heterochromatin.

3.2.2 MEIOTIC PREPARATIONS

- 1) At between 10.30 and 11.30am flower buds of the appropriate stage were fixed in freshly made-up 3:1 fixative and stored at 4°C until required (material kept in this manner showed little deterioration over 3 or 4 years).
- 2) Flower buds were dissected out and opened using a specially made multiple watch glass (great care was taken to keep all flower material on ice except when it was actually under the dissecting microscope)
- 3) Anthers were carefully slit open using fine tungsten needles in a drop of 2% aceto-orcein and the contents removed. The anther remains were discarded and a cover slip placed on the slide. Using the x30 magnification it was possible at this stage to discard PMCs that were too old or too young.
- 4) With a combination of gentle tapping and heating with a spirit burner the PMCs could be separated from their cell walls.
- 5) The slides were carefully squashed and the good ones sealed with rubber solution. Observations and photography were always done using these wet preparations.
- 6) If permanent preparations were required the cover slips were removed using the dry ice method, and the slides were passed quickly through two changes of absolute alcohol and mounted in Euparal.

3.2.3 FEULGEN MICRODENSITOMETRY

- 1) Roots were collected at midday to ensure a high mitotic index.
- 2) Roots were fixed in 4% neutral formaldehyde for 2 hours, washed well with several changes of water, and refixed in 3:1 absolute alcohol:glacial acetic acid.
- 3) Root-tips were stored at 4°C until required.
- 4) A batch of material including Allium cepa L. roots was hydrolysed for 60 minutes at 20°C in 5N HCl.
- 5) The root-tips were rinsed in distilled water.
- 6) The root-tips were stained in Feulgen reagent for 2 hours at room temperature in the dark.
- 7) The roots were rinsed in SO₂ water.
- 8) Squashes were made on clean unsubbed slides in 45% acetic acid.
- 9) The cover-slips were removed by the dry-ice method, and the slides put through two changes of 100% ethanol, mounted in Euparal and stored in the dark until required.
- 10) Measurements were made using a Vickers M85 Scanning microdensitometer set as follows: x40 objective, wavelength 540nm; spot size 2; band width 50; and scanning area x and y set to 1.

For reasons discussed later it was found necessary to use late prophase or metaphase nuclei for measuring purposes. Between 30 and 50 cells were measured in duplicate for each collection in each batch, an Allium control being included in each batch. The mean 4C value was then calculated and

converted to pg DNA by means of the Allium control. Slides of Rumex acetosa (female) and Vicia sativa L. were included as double checks in one of the staining batches.

3.2.4 GIEMSA BANDING TECHNIQUES

3.2.4.1 Preparation of air dried slides for banding,
modified version of Jamieson G. et al. (1986)

- 1) Collection and pretreatment was as in mitotic methods.
- 2) Roots were fixed either in 3:1 (3 hours to 3 weeks) or 4% neutral formalin.
- 3) Fixative was removed and the roots thoroughly rinsed in distilled water.
- 4) Roots were incubated for 45-90 minutes at 37°C in a mixture of 14 units ml⁻¹ Pectinase (Sigma P-5146) and 1% cellulase (Boehringer, Trichoderma viride) made up in 0.1M sodium citrate.
- 5) The enzyme solution was removed and the roots rinsed in water and transferred to 45% acetic acid.
- 6) Squashes were made on clean uncoated microscope slides and cover-slips.
- 7) Cover-slips were removed using the dry ice or liquid nitrogen methods.
- 8) Preparations were allowed to air-dry and were stored in a dust free area until required.

N.B. Earlier banding runs were done without the enzymic hydrolysis stage.

3.2.4_{ii} Newton's Technique

- 1) Air dried preparations were made as already detailed.
- 2) Slides were placed in 0.2N HCl for 1 hour in a coplin jar.
- 3) Water baths were prepared: a) 50°C with Coplin jars of 5% Ba(OH)₂ 8H₂O. (prepared on a stirring hot plate until dissolved, poured into an equilibrated Coplin jar at 50C, then filtered in a second equilibrated Coplin jar) b) 60°C with Coplin jars of Hanks solution (see materials section). Coplin jars were left to equilibrate whilst the slides were in 0.2N HCl.
- 4) The slides were transferred quickly without rinsing to the Ba(OH)₂ 8H₂O solution for approximately 3 minutes.
- 5) Ba(OH)₂ 8H₂O was quickly washed out with cold running tap-water.
- 6) The slides were transferred to Hanks solution for 1 hour at 60°C.
- 7) The slides were rinsed gently with tap-water, and stained in 2% Giemsa (Gurr improved R66 solution) made up in 6.8 buffer (Gurr) for 10-20 minutes.
- 8) Stain was rinsed out using buffer, and the slides allowed to dry and mounted in Euparal.

Modifications

- 1) Euparal mounting was soon abandoned as it tended to decolourise the preparations and subsequent preparations were examined directly.

- 2) In the absence of an enzymic hydrolysis stage a hot (60°C) 45% acetic acid treatment was sometimes used.
- 3) HCl hydrolysis time was varied from 60 to 70 min, was omitted, concentrations of between 0.1N and 0.4N were used, and on one occasion 5N HCl for 10 min was used.
- 4) Barium hydroxide solution was applied for 2.5 to 6 min at 52°C, and 3 to 8 min at 60°C.
- 5) Hanks solution treatment, varied between 1 and 1.5 hours.
- 6) Staining varied from 10 to 20 min with or without a xylol decolourisation stage.

3.2.4_{iii} Hutchinson's Technique (Personnel communication)

- 1) Air-dried preparations were made as previously detailed.
- 2) Acid hydrolysis was carried out in 0.1M HCl at 35°C for 50 min.
- 3) Ba(OH)₂ solution was prepared by adding 100ml distilled water at 52°C to 5gm of Barium hydroxide and mixing well.
- 4) Ba(OH)₂ solution was poured immediately over the slides and incubated at 35°C for 7.5 min.
- 5) Ba(OH)₂ solution was replaced with cold running tap-water until a clear solution was obtained.
- 6) Slides were transferred to 2xSSC for 1 hour at 52°C,
- 7) Slides were rinsed in distilled water.
- 8) Preparations were stained in 2% Giemsa in pH 6.8 buffer.
- 9) Slides were rinsed in distilled water, dried, and mounted in immersion oil.

Modifications

- 1) Fixation in both 3:1 and 4% formalin was used and on one occasion live root-tips were pretreated in enzyme mixture following the technique of Ruiyang et al. (1982).
- 2) The hydrolysis in 0.2M HCl at 37°C was sometimes omitted, especially if an enzymic hydrolysis stage had been included.

- 3) the $\text{Ba}(\text{OH})_2$ solution step varied between 4 and 15 min duration.
- 4) 2xSSC as per schedule, but on one occasion 4xSSC at 52°C for 60 min was used.
- 5) Staining was for 4-10 min in 2-4% Giemsa.

3.2.4_{iv} Technique of Schwarzacher et al. (1980)

- 1) Air dried preparations made as previously detailed.
- 2) 45°C acetic acid was applied for 15 min at 60°C
- 3) Acetic acid was washed out and rinsed with tap-water for 15-30 min.
- 4) Saturated $\text{Ba}(\text{OH})_2$ was added for 5-15 min at room temperature.
- 5) Slides were washed for 15-30 min in tap-water with a final rinse in distilled water.
- 6) 2xSSC was applied at 60-65°C for 1-2 hr.
- 7) Slides were rinsed with tap-water, then distilled water.
- 8) Preparations were stained in 3-4% Giemsa for 10 to 60 min and then washed very briefly in distilled water.

Times of 1 hr and 2 hr 10 min were used for stage 6.

3.2.5 FLUORESCENT STAINING

3.2.5₁ Materials

- 1) Chromomycin A3 (Sigma) 0.2mg/ml solution was made by dissolving in a minimal amount of ethyl alcohol and making up to volume with McIlvaine's citric acid- Na_2HPO_4 buffer with 10mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.
- 2) DAPI (Sigma) stock solution of 5mg/ml was prepared by dissolving the DAPI in a minimal amount of ethanol and making up to volume with McIlvaine's pH 7.0 buffer. Stock solution was stored in refrigerator until required and diluted 1/25 to give working strength of 0.2ug/ml.
- 3) Actinomycin D. (Sigma) stock and working strength concentrations of 0.2mg/ml were prepared by dissolving in a minimal amount of methanol and making up to volume with McIlvaine's pH 7.0 buffer.
- 4) McIlvaines pH 7.0 buffer
 Solution A 0.1M citric acid (4.2gm/200ml)
 solution B 0.2M Na_2HPO_2 (28.39gm/litre)
 185ml of solution A was mixed with 815 ml of solution B.
 When required 1.015gm/500ml of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was added to give 10mM concentration.
- 5) Mountants a) glycerol:McIlvaines pH 7.0 buffer 1:1 mixture
 b) 100mg p-phenylenediamine dihydrochloride (Sigma) was

dissolved in 10ml phosphate buffered saline. pH was adjusted to 8 with 0.5M carbonate-bicarbonate buffer (0.42g NaHCO_3 in 10 ml water, pH to 9 with NaOH). The solution was added to 90ml glycerol, filtered and stored at -20°C with the DAPI counterstain (Johnson 1981).

3.2.5_{ii} DAPI/ACTINOMYCIN D (Schweizer 1976)

- 1) Air dried preparations were kept in a refrigerator in the dark for a minimum of 2 days.
- 2) Pre-incubation was carried out in McIlvaines pH 7.0 buffer for 10 min at room temperature in a Coplin jar.
- 3) Buffer was replaced by 0.2ug/ml DAPI in McIlvaines buffer in the dark.
- 3) Slides were rinsed with buffer, placed in a moisture chamber with a drop of 0.2mg/ml Actinomycin D, and covered with a cover-slip for 15 min in the dark. They were mounted and viewed in buffer/glycerol mixture, sealed with rubber solution (better after 24hr storage in a refrigerator)
- 5) Slides were examined using a Zeiss standard microscope with the 2 FL vertical illuminator system fitted with an HBO 50W high-pressure mercury source; filter set 487702 for UV excitation.

3.2.5_{iii} DAPI/Chromomycin A3 Staining

- 1) Air dried preparations were incubated in 0.2ug/ml DAPI in pH 7.0 McIlvaines buffer for 15 or 60 min in the dark.
- 2) Slides were rinsed in buffer and placed in the dark in a moisture chamber with a drop of 0.2mg/ml chromomycin in pH 7.0 McIlvaines buffer with 10mM MgCl₂ under a cover-slip.
- 3) Slides were rinsed in the above buffer and mounted either in glycerol/buffer mixture or in the antifade

buffer.

- 4) Slides were examined using the previously described U.V. set up with the two filter sets 487702 (U.V. excitation) and 487709 (Blue excitation). Suitable metaphase plates were located using phase contrast and then examined with the appropriate excitation wavelength in an attempt to cut down on the fairly rapid fading that occurred. By having two filter sets in a 2FL holder it was possible to observe the same cell sequentially under both the excitation conditions.

3.3 RESULTS

3.3.1 CHROMOSOME NUMBER

Table 3.3 details all published counts of my taxa with the exception of F. convolvulus and F. dumetorum where the extensive literature has not been cited, due to its consistency and its only peripheral interest to this study. Table 3.4 gives a summary of my own counts and the number of accessions examined for each taxa; full details of these may be found in Appendix 1. Plates 3.1 to 3.4 show selected mitotic preparations from all the taxa examined.

My results add no new counts to the literature and are generally in agreement with the published counts, though I have been unable to confirm the $2n=20$ cytotypes of F. convolvulus and F. cilinodis. I have not found a single tetraploid R. japonica var. japonica in the British Isles, and all 33 octoploid British accessions examined have been male-sterile. A male-fertile clone from America P169 is the only male-fertile octoploid that I have seen. The only accession that I have from China is an octoploid, and , of the 4 collections received as seed from Japan, 3 have been tetraploids and one octoploid.

3.3.2 MEIOSIS

Plates 3.9 to 3.12 show representative meiotic preparations of the taxa investigated. The preferred^r stage for meiotic analysis in this material is diakinesis because earlier

stages tend to be very indistinct and at metaphase in polyploids the chromosomes are usually too crowded together on the spindle (Plate 3.12e). Table 3.5 summarises the meiotic analyses that have been carried out. It will be noted that no results are presented for either F. multiflora or the 8x Reynoutria japonicas. In the case of F. multiflora no flowers have been produced on either of the accessions in spite of their being maintained in a number of different growth environments. The octoploid R. japonica plants are, with the exception of the American accession P169 (which has not yet flowered at Leicester), male-sterile. Examination of the developing anthers of these female plants has shown that development ceases before meiosis. The alternative of looking at female meiosis has not been given serious consideration since only one meiocyte is present per flower, and even in good preparations of Reynoutria Pollen Mother Cells many hundreds need examining before an interpretable cell is located.

The results are very much what one would expect from a group of sexually reproducing plants. They are generally good bivalent producers although occasional multivalents are found in the tetraploid Reynoutria, and the clone of F. convolvulus examined seems regularly to have one multivalent (see Plate 3.10). Plate 3.9c illustrates some of the technical problems. It shows an F. dumetorum anther at the correct stage for meiosis; it is 0.2mm long and considerable patience was required with such anthers, particularly as they contained only about 6PMCs per locule.

3.3.3 KARYOTYPING

Karyotypes and karyograms are presented in Figures 3.1 to 3.8, and DNA-corrected karyograms in Figure 3.9; Table 3.1 gives a summary of some of the more important data used in the production of the karyograms. Examination of the figures immediately shows the different karyotypes to be uniform in terms of centromere position and chromosome length. Using the terminology of Levan et al. (1965) 59.8% of chromosomes are metacentric, 37.5% submetacentric and 2.67% subacrocentric, the last of variable occurrence between preparations of the same accession. Chromosomes are on average 2-2.5 μm with a range from 1.5 to 4 μm depending on their degree of condensation. The differential condensation of chromosomes both between and within accessions in these plants probably stems from the fact that my pretreatment schedules were developed primarily to cope with the octoploid R. japonica, where a very high degree of condensation is required in order to count the very numerous chromosomes. These techniques, when subsequently applied to diploid taxa, also had the effect of producing heavily condensed chromosomes. However less condensed chromosomes were also present but due to the lower chromosome number were suitable for karyotype use. By expressing all the chromosome arm lengths as a percentage of total 2x karyotype length similar karyograms could be produced from two cells of the same accession varying considerably in their degree of condensation (see Figures 3.1 and 3.2). The use of less highly condensed karyotypes is to be preferred since more accurate measurements may be obtained from them. However,

since it is impossible to obtain karyotypable preparations of polyploid Reynoutrias without a very high degree of condensation one is faced with something of a dilemma. One consequence of this is that there is not a good correlation between karyotype length and DNA C-value.

In spite of the general uniformity of these karyotypes in terms of size variation both within and between karyotypes, and the generally median to submedian centromere position, there are some noticeable trends. F. cilinodis has a marginally more symmetrical karyotype with an average arm ratio of 1.34, whilst the F. baldschuanica accessions tend to be slightly more asymmetrical than average with mean arm ratios between 1.6 and 1.9. Another general trend is that satellites when visible, are always on the short arm of one of the most asymmetrical chromosomes.

Taxon	Chr. No.	No. cells	shortest 2x karyo -type μm	longest 2x karyo -type μm	mean karyotype length μm	longest chr. minus shortest chr. μm	arm ratio least sym. chromosome	mean arm ratio	2x C value pg
<u>F. cilinodis</u> P148	22	2	49.9	59.8	54.8	0.68	2.45	1.34	2.19
<u>F. baldschuanica</u> P163	20	2	52.16	69.9	60.9	1.13	3.14	1.83	1.96
<u>F. baldschuanica</u> P152	20	2	74.83	85.76	80.2	1.57	3.7	1.9	-
<u>F. baldschuanica</u> P151	20	2	49.9	59.5	54.7	0.78	3.19	1.87	-
<u>F. baldschuanica</u> P175	20	4	45.08	59.8	49.72	0.72	2.66	1.6	-
<u>R. japonica v. jap.</u> P134b	44	1	-	-	93.8	0.45	2.62	1.7	1.41
<u>F. multiflora</u> P162	22	4	48.13	57.2	52.79	0.51	3.13	1.8	1.41
<u>R. sachalinensis</u> P155	44	1	-	-	95.8	0.72	2.29	1.58	1.35
<u>R. jap. v. comp.</u> P99a	44	1	-	-	101.4	0.82	2.15	1.69	1.29
<u>F. convolvulus</u> P150	40	2	43.2	48.14	86.5	1.15	3.32	1.43	0.72
<u>F. dumetorum</u> P177	20	2	30.0	51.47	40.73	0.77	2.19	1.47	0.68

TABLE 3.1 KARYOTYPE MEASUREMENTS

Taxon	Batch	Machine Units				Pg DNA	
		2c Mean	S.D.	n	Agg. 2c mean	2c DNA mean	DNA per 2x genome
<u>F. dumetorum</u> 2n=20 P177	E	7.83	1.35	35	7.83	0.68	0.68
<u>F. convolvulus</u> 2n=40 P150	E	16.6	2.9	32	16.6	1.44	0.72
<u>R. japonica</u> var. <u>japonica</u> 2n=88 P35	A	51.9	4.9	43			
	A	41.1	3.95	28			
	A	47.5	5.17	50	47.6	4.36	1.1
<u>R. japonica</u> var. <u>compacta</u> 2n=44 P2A	B	25.6	3.09	30			
		26.4	3.65	26	26	2.57	1.29
<u>R. japonica</u> var. <u>japonica</u> 2n=44 P114b	B	25.5	2.9	25			
		27.1	3.2	25	26.3	2.59	1.30
<u>R. sachalinensis</u> 2n=44 P171	C	31.5	3.4	24	31.5		
	E	31.9	3.7	34	31.8	2.65	1.33
<u>F. multiflora</u> 2n=22 P162	E	17.1	1.8	14			
		15.9	2.8	32	16.3	1.41	1.41
<u>R. japonica</u> var. <u>japonica</u> 2n=44 P172	A	35.3	4.1	49			
		32.2	4.7	41			
		32.1	3.7	43	33.3	3.06	1.53
<u>R. japonica</u> var. <u>japonica</u> 2n=88 P169	C	79.8	7.5	34			
		81.1	8.8	31	80.4	6.48	1.62
<u>F. baldschuanica</u> 2n=20 P163	A	22.2	3.3	35	21.4	1.96	1.96
		20.8	3.6	49			
<u>F. cilinodis</u> 2n=22 P156b P156b P156b P148 P148	A	23.9	2.7	37			
	A	22.8	3.2	24	23.5		
	B	24	2.4	41	24		
	C	26.3	3.1	25	26.3		
	D	23.4	2.6	35			
	D	22.3	3.1	24	23	2.19	2.19

TABLE 3.2 MICRODENSITOMETRY RESULTS

Species	Chr. No.	Author and Date	Origin of Plant
<u>F. multiflora</u>	n=11	Sugiura, T. 1936	not given
<u>F. multiflora</u>	2n=22	Suzuka, O. 1950	not seen
<u>F. multiflora</u>	2n=22	Doida, Y. 1960	Higashiyama Botanic Gardens Nagoya Japan
<u>F. baldschuanica</u>	2n=20	Jaretsky, R. 1928	not seen
<u>F. baldschuanica</u>	2n=20	Schnack, B. Fernandes, O 1946	not seen
<u>F. cilinodis</u>	2n=20	Jaretsky, R. 1928	not seen
<u>F. cilinodis</u>	2n=20	Löve, A., Löve, D. 1964	Manitoba; Canada
<u>F. cilinodis</u>	2n=20	Löve, A., Löve, D. 1982	Manitoba; Canada
<u>F. cilinodis</u>	2n=22	Kapoor and Gervais 1982	Nova Scotia; Canada
<u>R. sachalinensis</u>	2n=C44	Jaretsky, R. 1928	Kieler Botanic Gardens Germany
<u>R. sachalinensis</u>	2n=44	Sinotô, Y. 1929	not seen
<u>R. sachalinensis</u>	2n=C66	Menshikova 1964	not seen
<u>R. sachalinensis</u>	2n=44	Sokolovskaya 1960	Ullungdo Island; Korea
<u>R. sachalinensis</u>	2n=102	Lee, Y.N. 1972	Poland
<u>R. sachalinensis</u>	2n=44	Wcislo, H. 1977	Hamburg; Germany
<u>R. japonica</u> var. <u>compactum</u>	2n=44	Jaretsky, R. 1928	Kieler Botanic Garden Germany
<u>R. japonica</u> var. <u>japonica</u>	2n=C88	Jaretsky, R. 1928	Sinano province: Japan
<u>R. japonica</u> var. <u>japonica</u>	n=22	Sugiura, T. 1931, 1936	Russia
<u>R. japonica</u> var. <u>japonica</u>	>60	Zhukova, P.G. 1967	Misima; Japan
<u>R. japonica</u> var. <u>japonica</u>	2n=44	Doida, Y. 1960	Seoul; Korea
<u>R. japonica</u> var. <u>japonica</u>	2n=88	Lee, Y.N. 1972	Czechoslovakia
<u>R. japonica</u> var. <u>japonica</u>	2n=44	Majovsky, et. al. 1974	Czechoslovakia
<u>R. japonica</u> var. <u>japonica</u>	2n=44	Murin, A. 1974	Poland
<u>R. japonica</u> var. <u>japonica</u>	2n=88	Wcislo 1977	China
<u>R. japonica</u> var. <u>japonica</u>	2n=52	Liu, Y.	
<u>F. cynanchoides</u>		cytologically unknown	
<u>F. scandens</u>	2n=34	Smith 1963	Kansas; U.S.A.
<u>F. scandens</u>	2n=20	Löve, A. Löve, D. 1982	Manitoba; Canada
<u>F. dumetorum</u>	2n=20	numerous reports	
<u>F. convolvulus</u>	2n=20	numerous reports	
	2n=40	numerous reports	

TABLE 3.3 PUBLISHED CHROMOSOME COUNTS OF REYNOUTRIA AND FALLOPIA

Species	Chr. No.	No. Counted	Origin of Accessions
<u>R. japonica</u> var. <u>japonica</u>	44	3	All from native Japanese material
<u>R. japonica</u> var. <u>japonica</u>	88	38	1 Japanese, 1 Chinese, 1 American, remainder from the British Isles.
<u>R. japonica</u> var. <u>compacta</u>	44	4	All from the British Isles.
<u>R. sachalinensis</u>	44	24	2 Japanese accessions, others from British Isles.
<u>F. cilinodis</u>	22	4	Canada (wild + cult.) and Warsaw Bot. Garden.
<u>F. baldschuanica</u>	20	5	1 Chinese plant, rest garden origin.
<u>F. convolvulus</u>	40	3	1 Italian, the others British.
<u>F. dumetorum</u>	20	2	1 British, the other from Sweden.

TABLE 3.4 SUMMARY OF COUNTS CARRIED OUT AT LEICESTER

Taxon	Chr. No.	No. Cells	MEIOTIC Configurations						Mean Chiasma frequency per cell	Mean Chiasma frequency per bivalent
			Is	Rod IIs	Ring IIs	IIIs	3xta IVs	4xta IVs		
<u>F. dumetorum</u> P177	20	3		19	11				13.67	1.37
<u>F. baldschuanica</u> P174	20	19		150	40				12.11	1.21
<u>F. cilinodis</u> P148	22	5	1	32	21	1			15.2	1.38
<u>F. convolvulus</u> P178	40	7		92	36		6		26	1.30
<u>R. japonica</u> var. <u>compacta</u> P2	44	23	4	416	79	2	3		25.52	1.16
<u>R. japonica</u> var. <u>japonica</u> P114b	44	7		125	29				26.14	1.18
<u>R. sachalinensis</u> P68	44	13		208	70		2	2	27.69	1.26

TABLE 3.5 SPECIES MEIOTIC ANALYSIS

3.3.4 MICRODENSITOMETRY

A summary of results is set out in Table 3.2; the same data are graphically represented in Figure 3.11, and DNA-corrected karyotypes are shown in Figure 3.9. Table 3.2 sets out the 2C means and standard deviations in arbitrary units, as well as the 2C DNA values in pg per nucleus and per 2x genome. Values for the Allium standards are not provided in the table but may be inferred from the conversion factor that accompanies each of the different batches.

Turning first to the total 2C DNA values, the octoploids have, not surprisingly, the highest absolute values (between 4.4 and 6.5pg). I am a little surprised by this degree of variation since it is much greater than that found amongst the tetraploids. One explanation could be that the P35 plant used was a little too pot-bound and that the root-tips as a consequence carried a higher level of tannins which in turn depressed the measured DNA value. The tetraploid Reynoutria taxa are fairly consistent, with values of between 2.54 and 3.06pg. The diploids F. baldschuanica and F. cilinodis have between 1.96 and 2.19pg and thus closely approach the lower limit of the tetraploid Reynoutria values, and significantly exceed the 1.41pg of the tetraploid F. convolulus. It is gratifying to note that, in spite of the difficulties of using metaphase preparations and the consequent high standard deviations, some quite reasonable interbatch correspondences were achieved. Notably, the F. cilinodis values for batches A, C and D show

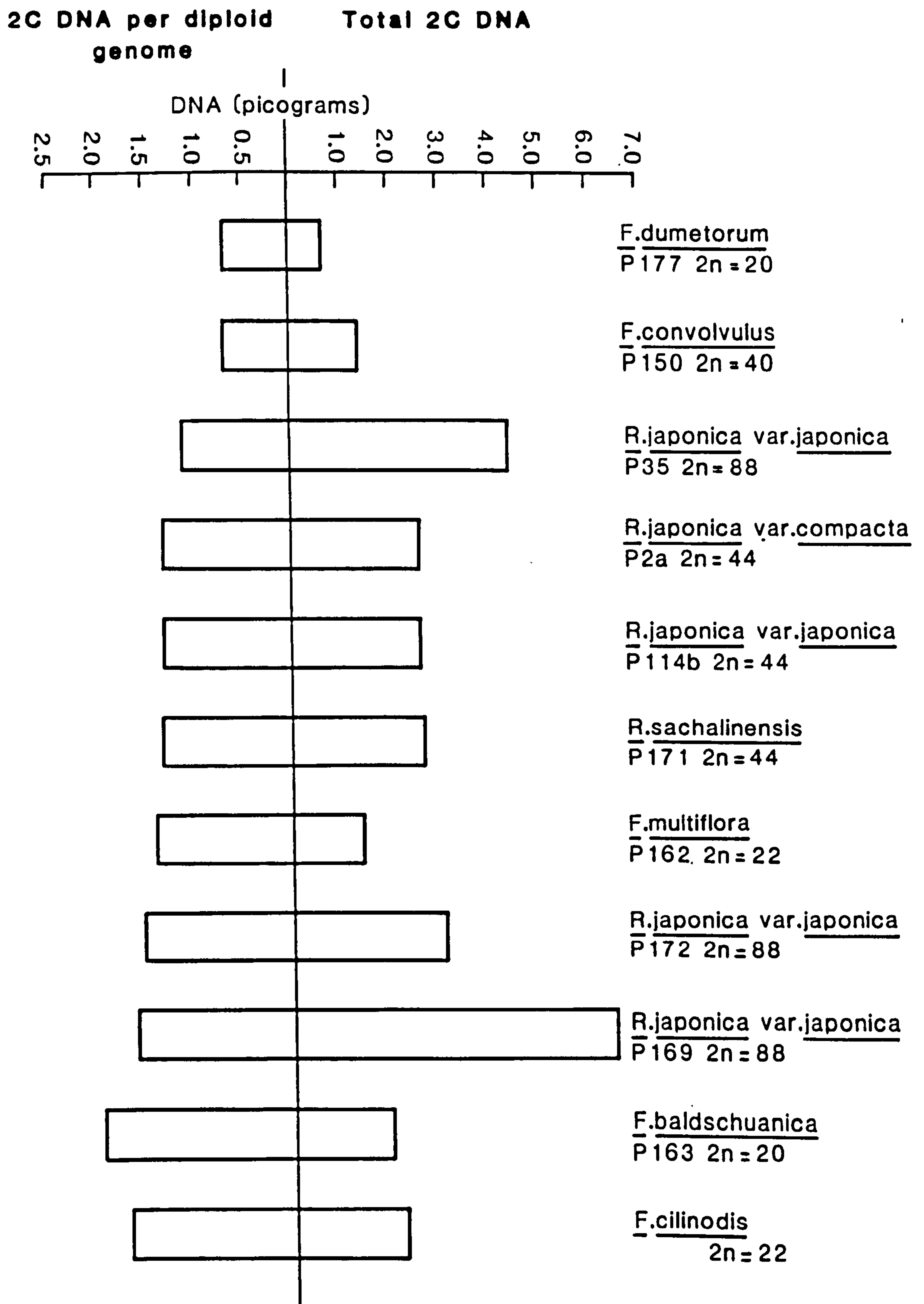


Figure 3.11 DNA C value of *Reynoutria* and *Fallopla* species

very good consistency, and R. sachalinensis in batches C and E at 2.54 and 2.76pg are quite respectable. The diploid F. multiflora has the second lowest C-value, followed by that of the annual diploid F. dumetorum.

Ranking the C-values in order of 2x DNA value radically changes this order and, it could be argued, splits the taxa into groups. Highest 2x DNA values are found in the diploids F. cilinodis and F. baldschuanica, followed by the octoploid and tetraploid Reynoutria and F. multiflora. There is then a sharp drop in C value to the two annuals F. dumetorum and F. convolvulus.

3.3.5 CHROMOSOME BANDING

With regard to chromosome banding of Reynoutria and Fallopia taxa I have very little to report since I have had little success. Plates 3.5 to 3.7 refer to banding, but most of these feature Hyacinthoides hispanica and Rumex acetosa which were included in the various staining runs as positive controls. Plate 3.7 of H. hispanica is particularly interesting since it shows an example of complete correspondance between Giemsa C-bands and chromomycin-bright material. The Giemsa-positive chromocentres visible at interphase are also chromomycin-bright at interphase. Since chromomycin is a specific stain for G-C regions, it follows that in H. hispanica the C-bands are G-C rich. No DAPI or chromomycin enhanced or quenched regions were observed in any of the Reynoutria and Fallopia cells examined (Plates 3.6a-c, 3.8a).

Unfortunately I was unaware of the literature relating to the heterochromatic nature of the chromosomes of Rumex acetosa until after I had finished my attempts at banding, and I have subsequently had time for only a brief examination of it. One advantage of this plant is the great ease with which one can obtain karyotypable metaphase plates, a great contrast to Reynoutria species. This lack of a thorough examination of R. acetosa is regrettable since it would have been a more relevant control for my batches of banding than the Hyacinthoides that was used. Indeed it could be argued that the use of an inappropriate positive control is of no use, since in an exacting treatment such as

Giemsa banding there is no certainty that the appropriate treatment for one taxa will also be useful for a completely unrelated one.

Plate 3.5 shows some pictures of the sort of linear differentiation that I was able to find in Reynoutria and Fallopia; Plate 3.5a is stained with aceto-orcein, 3.5b with Giemsa, and 3.5c is an unstained wet mount viewed by phase contrast. This differentiation was most obvious from mid to late prophase, but by C-metaphase it was generally very difficult to see. Comparison may be made between the C-banded preparation shown in Plate 3.5b and the DAPI-stained cell in 3.6a. Plate 3.6b and c show the same cell stained with DAPI and chromomycin respectively; no obvious differences are apparent.

My results with the fluorescent-banding of R. acetosa are broadly in line with those of Leeman and Ruch (1983). Plate 3.13 shows 2 pictures of the same cell double-stained with the fluorescent agents DAPI and chromomycin A3. Comparison of the two photographs shows that the chromomycin-bright regions (arrowed) are totally quenched in the DAPI stained preparation. Further, the Y-chromosomes (arrowed) show slightly diminished fluorescence with chromomycin and slightly enhanced fluorescence with DAPI, which indicates that they are relatively rich in A-T. Leeman and Ruch also found two smaller DAPI-positive chromocentres. The two chromomycin-bright areas probably correspond to the regions adjacent to the nucleolar organizer, which according to Schweizer is a common phenomenon.

3.4 DISCUSSION

3.4.1 CHROMOSOME NUMBER

Within the genera Fallopia and Reynoutria there exist clear differences in the relative occurrence of polyploids and diploids. Fallopia contains a single polyploid (F. convolvulus), and the base numbers of the remaining diploids are 10 and 11 with F. cilinodis purported to exist at both levels, though I have only seen the latter. Reynoutria is entirely polyploid with a base number of 11. Anomalous or erroneous counts are reported for R. sachalinensis $2n=106$ (Lee, 1972), R. japonica $2n=52$ (Liu 1985), and F. scandens $2n=34$ (Smith 1963); these are excluded from any discussion of base number and ploidy level.

A significant number of reports date from the 1920s and 30s, and it is to the credit of those early investigators working on unpretreated material and with less sophisticated microscopes that they obtained such accurate results from these small numerous chromosomes. A notable omission, and one that I have failed to fill, is that of F. cynanchoides which is still cytologically undescribed. Few of these reports give more than a number and a locality (some do not even give the latter); some present line drawings and Lee provided some photographs, but no one has presented karyotypes.

Whilst polyploidy is the rule in Reynoutria it is restricted in Fallopia to section Fallopia; where the ubiquitous

agricultural weed F. convolvulus has both diploid and tetraploid cytotypes. Tetraploid annuals are not very commonly found, though the undoubted success of the 4x F. convolvulus may point to an advantageous trade-off between the increased load caused by the DNA doubling and the increased heterozygosity and tolerance of inbreeding depression consequent to it. R. sachalinensis is represented only at the tetraploid level, since Menshikova's (1964) count of C.66 is probably a hybrid with R. japonica. R. japonica is represented at both tetraploid and octoploid levels, the count of >60 (Zhukova 1967) could be an inter-chromosome race cross, or a R. japonica x R. sachalinensis hexaploid.

It would be very interesting to know if there are any geographical or ecological correlates of this difference in ploidy level, and in this the literature is singularly unhelpful. Unfortunately the greater part of the counts have been carried out on material naturalized in Europe or from Botanic gardens, and if the authors were aware of the original native origin on the whole they did not consider it important enough to communicate. Consequently, all that we can say with certainty about the native distribution of octoploid and tetraploid R. japonica is that tetraploids and octoploids are found in Japan and that an octoploid occurs in South Korea. There is a complete dearth of Chinese counts apart from the $2n=52$ of Liu; further details of this cannot be obtained since the full reference has been omitted from the chromosome report. Attempts to obtain seed from native localities in China have met with no success. The

only chinese Reynoutria that I have is an octoploid from Peking Botanical Garden and of unknown origin. The Japanese have been more forthcoming and I have obtained seed from several localities (see Appendix 1). The results in this section constitute the first detailed study of these two genera since Jaretzky in 1928.

3.4.2 MEIOSIS

Probably as a result of the tannin content of the inflorescences I found it very difficult to obtain good clear preparations of meiocytes. F. baldschuanica and F. cilinodis (Plate 3.9) were the exceptions to this and good contrasty preparations could be obtained in which it was easy to distinguish rod from ring bivalents and even to see the position of the chiasmata. Generally speaking, though (Plate 3.12 a and b) the appearance of the Reynoutria bivalents is somewhat diffuse. This diffus^e_{ness} is probably analogous to that found in the majority of Reynoutria mitotic preparations, where it obscures the precise position of the centromere.

It must of course, be acknowledged that the stage for most accurate assessment of chiasma frequency is late pachytene or diplotene and that the scoring of chiasma frequency at prometaphase can, particularly in long chromosomes, lead to an underestimate of the frequency. Large numbers of small, poorly stained chromosomes effectively rule out this approach for my taxa and so one has to make do with analysis at prometaphase. Even this is not without problems since in practice it is very difficult to distinguish ring bivalents from rod bivalents. Plate 3.9e, for example, illustrates this well; I have scored this as containing only 3 ring bivalents since my criteria for a ring bivalent are that not only should the chromosomes be lying side by side but that these should be clearly joined at both ends. I suspect that the plane in which a bivalent is lying at the time of

squashing may drastically affect its appearance; lying in one plane the pressure of squashing may force apart the arms of a rod bivalent, whilst the same bivalent lying in a different plane may end up with the two halves lying side by side as in a ring bivalent. Plate 3.9e shows rod bivalents in various different orientations including one with one half of the bivalent coming up out of the plane of the paper. Bearing this in mind, plus the relatively small number of cells analysed, one must regard the chiasma frequencies presented in Table 3.5 as somewhat tentative.

Taking the average chiasmata frequency per bivalent it may be seen that the range of values for diploids and tetraploids is not very different. It must be noted that the range of chiasma frequencies per bivalent of 1.16 to 1.38, which is rather low, but this is not unusual in the case of small chromosomes. John and Lewis (1965) reported that in the grasshopper Chorthippus brunneus the number of chiasmata per bivalent was related to the length of the chromosome, the longest chromosome in the complement having 2 or 3 chiasma, medium sized chromosomes 1 or 2, and the smaller ones only 1. 'Small' in this instance was 3-6 μ m long.

It may also be noticed that the potentially in-breeding taxa F. convolvulus, F. dumetorum and F. cilinodis have the highest chiasma frequency, which is in agreement with results presented by Stebbins (1971) in tribe Hordeae (Triticeae) of the Poaceae, where there is an inverse relationship between degree of out crossing and chiasmata

frequency. Stebbins suggests that low recombination frequencies and predominant self fertilization are alternative strategies for maintaining adaptive gene combinations in situations where this has a high adaptive value.

Finally, if Jackson (1982) is correct and the first chiasma is not randomly distributed but allocated one per bivalent, it is possible that taxa with low chiasmata frequency are to a certain extent preadapted to polyploidy, in that the number of multivalents in a newly created autoploid or segmental allopolyploid would be reduced simply by a lack of chiasmata needed to form them. A rod bivalent requires 0.5 chiasma per chromosome whilst a rod trivalent needs 0.67 chiasma per chromosome. This aside, lack of a significant number of multivalents in the polyploid taxa points to some means of multivalent suppression, unless of course, these taxa arose as allopolyploids in which case multivalent suppression would not be necessary. Multivalent suppression is not completely understood but its control in the intensively studied wheat genome is by the Ph-gene on chromosome 5B.

In rhizomatous perennials such as the Reynoutrias even if the onset of polyploidy initially brings about partial or complete sterility there is still a chance that over the years accumulation of chance mutations may lead to some genome differentiation and ultimate bivalent formation. This would not be the case in an annual weed such as F. convolvulus, unless it was of allopolyploid origin or its

polyploidy preceded its weedy annual habit.

3.4.3 KARYOTYPING

From these results it can clearly be seen that in Reynoutria and Fallopia, chromosome evolution (apart from base number changes) has not kept pace with morphological evolution, and that generally a single chromosome taken from any other karyotype would not look very much out of place in any other karyotype (at the same degree of contraction). With chromosomes in excess of 10 μm it might make sense to follow Abraham and Prasad (1983) and divide chromosomes into nine different groups on the basis of centromere position. However, with chromosomes of this size and uniformity that approach would be patently ridiculous. Bentzer et al. (1971) listed a number of factors such as squashing pressure, degree of chromosome contraction, and measuring error, all of which have a bearing on the accuracy of the karyotypes produced. As far as chromosome contraction is concerned, Bentzer et al. concluded that, whilst this has a significant effect on the arm ratio of telocentric chromosomes and a lesser effect on subtelocentrics, the effect of different states of condensation had little effect on the arm ratios of chromosomes with an arm ratio of less than 3.0, which includes the great majority of Reynoutria and Fallopia chromosomes. Indirect support is given to this by Figure 3.12, which shows a linear relationship between total karyotype length and the size difference between the largest and smallest members of the complement.

It may be seen that, although the chromosomes are arranged in order of size, they are unnumbered; this is because I do

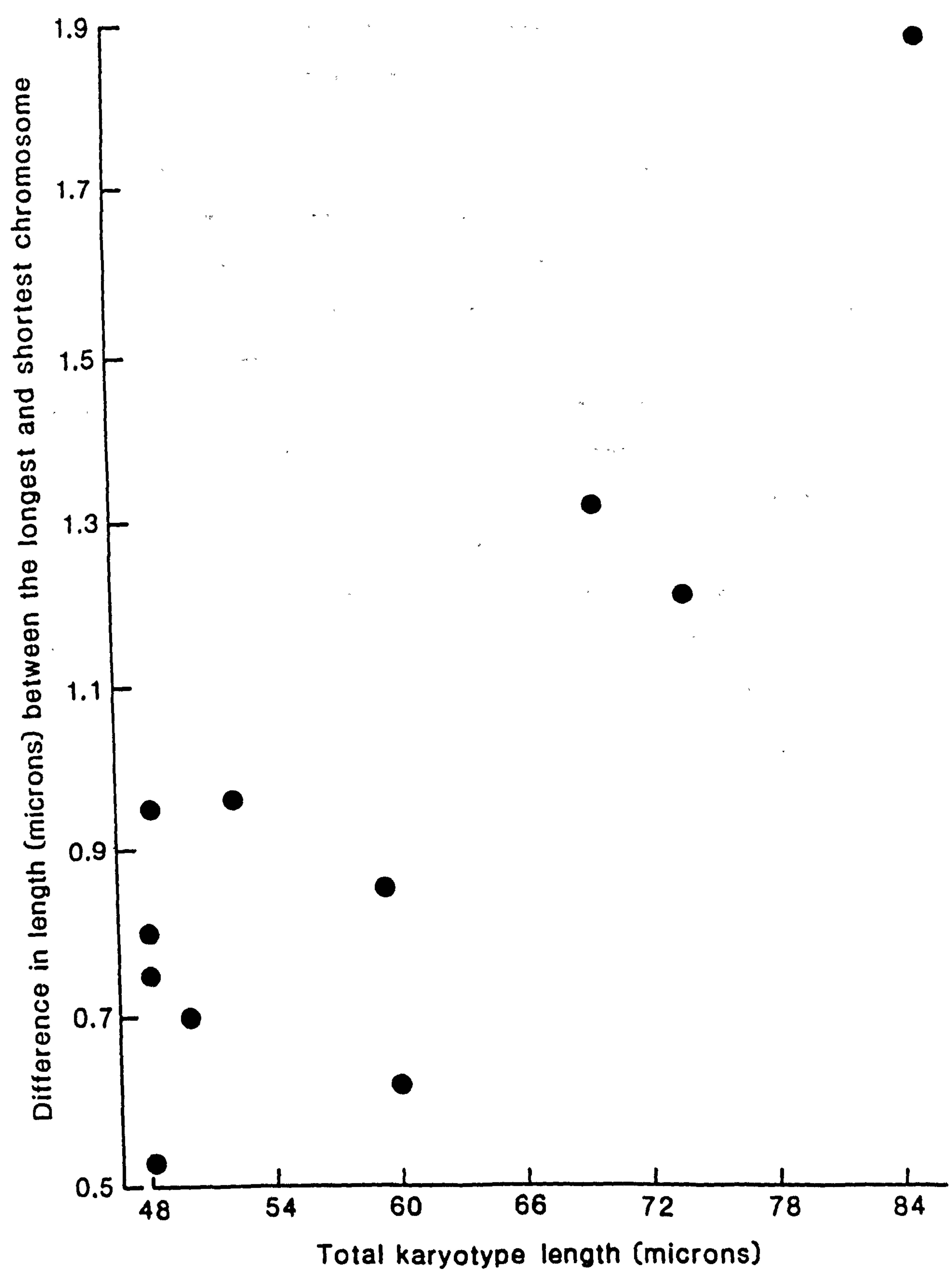


Fig 3.12 Graph showing the relation between karyotype length and the difference in length between the longest and shortest chromosomes in *F.baldschuanica*

not consider the data to be reliable enough to do this meaningfully. The cut-out chromosomes are generally less than 1 cm long (some are less than 6mm) and no matter how good the preparations are one is working very close to the limits. Further, with chromosomes of such uniform length a fraction of a millimetre can have drastic effects on the chromosome ranking; in one preparation of F. baldschuanica, for instance, 5 chromosomes are separated by a total length difference of 0.3mm, which is well within the error limits of the karyotyping process. Bentzer et al. also cited other researchers as stating that the danger of chromosome reversal (i.e. confusion of non-homologues) cannot be ignored in chromosomes with less than 11% length difference. Since few adjacent Reynoutria or Fallopia chromosomes differ by as much as 11% the case for meaningfully making interspecific karyotype comparisons appears very weak indeed.

3.4.4 BANDING

My interest in chromosome banding stems not only from the fact that it is a more sensitive approach than ordinary staining, but also because it is especially useful in the investigation of genome homologies amongst related taxa. This is particularly so if the karyotypes of such taxa are rather uniform and contain groups of chromosomes that are very difficult or even impossible to distinguish one from the other. The occasional presence of differential staining in some Reynoutria cells (Plate 3.5a) encouraged me to believe that the chromosomes could be banded. Accordingly I took some material to the laboratories of Jane Hutchinson and Martha Newton in Manchester and with their kind assistance I was able to get some encouraging results, in that it appeared that the chromosomes had the propensity for banding. Back in Leicester I tried to improve upon these results, firstly by varying those steps that were supposed to be species specific. Having exhausted these permutations with little or no improvement I started to experiment with the other steps in the procedure, still with little improvement, I found that whilst it was relatively easy to give too vigorous a treatment to Allium or Hyacinthoides resulting in breakdown of the chromosome structure, this proved exceedingly difficult in the case of Reynoutria and Fallopia, where extremely severe treatment still produced deeply stained well contracted metaphase chromosomes.

Initially my banding attempts were made using 3:1 (ethyl alcohol:glacial acetic acid) fixation, with softening prior

to squashing done with 45% acetic acid. Having seen Greilhuber's (1986) paper detailing the effect that tannins had on Feulgen staining, it occurred to me that this might also affect chromosome banding. The use of formalin rather than 3:1 fixation caused the tannins to be immobilised rather than permeating through the tissues. The use of formalin fixation with 45% acetic acid softening was not a success, since formalin has the unfortunate effect of excessively hardening the cell contents making squashing more difficult. In order to counteract this, a pectinase/cellulose step was introduced prior to squashing. This gave much improved results with formalin and 3:1 fixed roots. Even so, squashes with formalin-fixed material were still inferior to those fixed in 3:1. It was, however, noticeable that in formalin-fixed cells it was much easier to degrade the chromosomes with Barium hydroxide and 2xSSC, suggesting that the tannins in 3:1 fixed material were protecting the chromosomes from denaturation. Another problem with formalin-fixed cells was that the immobilized tannin bodies were hard and incompressible (see Plate 3.8a) further hampering the preparation of well spread metaphase plates.

Having reached stale mate with the Giemsa techniques, I then went on to try various fluorescent banding schedules. Assuming that these chromosomes did have the potential for banding, but that I had just failed to find the magic permutation, fluorescent banding promised a different perspective and a chance to test this assumption. Fluorescent banding offered two main advantages. Firstly,

no elaborate sequence of treatments preceded fluorescence staining, simple air-dried preparations being all that is required. Secondly, the technique is supposed to be remarkably sensitive, Schweizer (1980) claiming that between 0.01 and 0.005pg G-C-rich DNA may be detected using chromomycin and a counter stain; thus the small size of the Reynoutria chromosomes would not necessarily be a problem. Whilst Reynoutria and Fallopia chromosomes were readily stained by these fluorescent agents, they showed no signs of differential fluorescence, although in the control Hyacinthoides and Rumex acetosa slides I was able to see chromomycin-bright regions, and quenching as well as DAPI-enhanced fluorescence (Plate 3.13). The heterochromatin of R. acetosa will be dealt with in some detail since it is the only reported example in the Polygonaceae. Kurita and Kuroki (1970), in an examination of the course of prophase condensation, found that the Y-chromosomes consisted of one of the prominent interphase chromocentres or sex bodies plus a segment of euchromatin. At early prophase the chromocentre forms part of the short arm of the chromosome, the remainder of the short arm and the long arm being euchromatic. However, by mid prophase this euchromatin has all condensed out of synchrony with the X and autosomal chromosomes, leading to an entirely heteropycnotic chromosome at mid prophase. Whilst the chromosome is clearly heterochromatic in the classical sense, it does not follow that it is totally heterochromatic in the Giemsa C-banding sense. My own brief examination of C-banding of R. acetosa showed that bands were certainly not restricted to the Y chromosomes (Plate 3.13). Since the R.

acetosa examined had been subjected to 16 hr pretreatment in 800 in the refrigerator overnight, it is pertinent to consider something of the relationship of cold-induced segments, heterochromation and C-bands. The preparation shown in Plate 3.5c proved something of a turning point, since it is an unstained preparation with obvious linear differentiation. That this sort of preparation could be obtained without acid hydrolysis or any of the C-banding procedures cast serious doubt on the interpretation of earlier orcein and Giemsa preparations as being indicative of C-band potential. Although the cold treatment given to the root-tip was less severe than the 2-3 days in the cold used in the classical demonstrations of allocycly, the occurrence of cold-induced segments was indicated. If this were the case the preparations shown in Plate 3.5a, b and 3.6a would not be caused by the selective removal of DNA from certain regions, but would be a reflection of the different concentrations of DNA present along the chromosome caused by the late replicating negatively heteropycnotic segments of DNA. This would be no problem if the negatively heteropycnotic regions present in the prophase became C bands in the Giemsa stained metaphase, but the relationship between cold-induced segments and C-bands is not straightforward. Takehisa and Utsumi (1973), working on Trillium kamschaticum Pall., found that, although some cold-induced segments corresponded to C-bands, a major C-band was present in the long arms which was not visible as a cold-induced segment. Greilhuber (1979) conversely found in Adoxa that cold-induced segments exactly mirror the distribution of C-bands, but also cited his earlier research

in Vicia faba L. which revealed only a minority of the stained segments to be heterochromatic on the basis of C-banding. Clearly there are some little-understood interactions at work here. There is, of course, the possibility that C-bands were not found because the chromosomes simply do not possess any. Certainly none of the bands found in Reynoutria or Fallopia had the same clear contrast as was found in Hyacinthoides nuclei (Plate 3.7c) or in much of the published work, nor were such clear chromocentres seen in the interphase nuclei.

The fluorescent banding was carried out as a double check, in case the lack of fluorescent banding added weight to the notion that no C-bands existed. In order to do this one must know something of the relationship between the various types of fluorescent banding and C-bands for it is not always such a straight correlation as in Hyacinthoides (Plate 3.7). Schweizer (1982) reported that in human chromosomes G-C-specific chromomycin banding patterns correspond to R- or reverse-banding patterns whilst A-T-specific banding patterns correlate with Q- and G-like banding patterns. Such complementarity, he said, had been used to support the argument that R/Q banding patterns represent variations in DNA base-structure along the chromosome. In plants Schweizer reported poor correlation between C-bands and chromomycin brightness, but a very good correlation between chromomycin brightness and regions at or adjacent to the nucleolar organiser regions.

Deumling and Greilhuber (1982) working on Scilla siberica

Haw. where all the major C-bands were known to be G-C- rich, found a good correlation between C-banding and chromomycin brightness. However, with A-T- specific fluorochromes such as Quinacrine and DAPI, these same bands failed to show consistently reduced fluorescence. Deumling and Greilhuber concluded from this that characterisation of plant heterochromatin by fluorochromes is not an infallible indicator of base sequences present.

Thus the failure to demonstrate fluorescent banding cannot be taken as positive proof that C-bands are absent from these taxa, especially as there are suspicions that even with formalin fixation the tannins can still exert some sort of effect on the staining propensities of the chromosomes.

There is, of course, nothing intrinsically wrong with the use of cold-induced segments as a means of karyotype elucidation and this has been successfully done in some of the large chromosome taxa, such as Trillium (Darlington 1940) and Adoxa (Greilhuber 1979). It could be argued that banded karyotypes could be produced from some of the preparations I have shown. However, the task of marrying up diffuse late prophase heterochromatic preparations already produced is not only beyond my ability, but also a process that would so blur all karyotype distinctions as to be quite meaningless.

3.4.5 MICRODENSITOMETRY AND C-VALUE

Very few C-values have been published for the Polygonaceae, so it is rather difficult to make meaningful comparisons. Bennett and Smith (1976) presented DNA values for several Rumex taxa, but unfortunately these were expressed as 'per cell' values which are not directly comparable with C-values. Bennett, Smith and Heslop-Harrison (1982) gave 2 C-values of 3.4pg and 8.8pg respectively for the tetraploid Rumex acetosella and the octoploid R. crispus, both of which are rather higher than my results.

Whilst I am reasonably satisfied with the accuracy of my comparative DNA data, I am less so with my absolute DNA values reported. These doubts are heightened by results from other previously measured taxa that I included as double checks. When I was more than half-way through my DNA determinations it was suggested that I should include a standard whose DNA value was closer to that of my taxa. This was a sound point since the 2C DNA of the standard Allium cepa was five times higher than that of my highest value. The use of Allium also had the unfortunate effect of restricting me to the x40 objective of the microdensitometer since Allium nuclei are too large for the x100 objective. It is possible that, when measuring such small nuclei as those of Reynoutria at x40, the instrument was not being operated in the optimal part of its range. The extra controls used were a Rumex acetosa female and Vicia sativa, the latter having a published C-value of between 4.0 and 6.1pg (Bennett and Smith 1976). Finding the C-value for R.

acetosa was more involved, since, although Leeman and Ruch (1983) had published a 2C value of 7pg, this was for a male plant! R. acetosa has a constitution of 12AXX in the female and 12AXYY in the male. By using Wilby and Parker's (1986) figures indicating that Y_1 is 83% of the X and Y_2 74%, it was possible to arrive at an estimated 2C value for female plant of 6.57pg. My own values for R. acetosa of 4.17pg and 2.52pg for V. sativa are clearly underestimates. Much of the blame for this must go on the use of metaphase cells for measurement but it is not impossible that real differences were also involved since there is some regional variation amongst plants. Although at one time such variation was not thought to exist, Price (1988) lists several well-documented cases of intra-specific variation in DNA content. In the case of Allium I demonstrated that the use of late prophase and metaphase rather than early prophase resulted in a 22% underestimate of C-value. It was necessary to use metaphase and late prophase in Reynoutria since, unless the DNA was highly concentrated the nuclei did not take up the stain properly. Initial attempts at using early prophase cells led to readings that ranged between 10% and 60% of the metaphase readings, with several orders of magnitude of difference between the highest and lowest value, clearly of little use for DNA estimation purposes.

These staining problems probably stem from the presence of tannins, which the use of formaldehyde fixation had not entirely eliminated. A possibility that was not explored is the use of other plant tissue such as PMCs or leaf-tips in the hope that they contained fewer tannins. Methodological

considerations aside, reasonably reproducible results were obtained when the same accession was included in different batches. In any case, in terms of this research it is the comparative DNA results that are of more interest than the absolute DNA values.

What conclusions, if any, can be drawn about the pattern of DNA variation in this group of related taxa with its range of ploidy level, life-form and growth habit? In terms of $2x$ $2C$ value the annual diploids have the lowest values, Reynoutria and F. multiflora occupy the middle range, and the relatively unspecialized F. cilinodis and the woody perennial F. baldschuanica have the highest amounts. From these data it is not really possible to say with certainty whether amongst the herbaceous perennial taxa there is an increase or decrease in genome size with increasing ploidy level. Since the diploid F. multiflora falls amongst the tetraploids and the variation of the octoploids is high, it appears that no such differences exist. The annual taxa F. dumetorum and F. convolvulus clearly have the lowest DNA contents and it might be argued that this is linked to the annual habit. If as Bennett (1982) suggests, the minimum generation time is linked to the nuclear DNA content, a low C -value and fast minimum generation time is obviously advantageous to annual and ephemeral taxa.

The notion that small DNA values are more advantageous for annuals than perennials must of course be balanced against the need to maintain genetic heterozygosity, which might argue the need for a larger DNA amount for annual taxa.

Data published by Bennett and Smith (1976) lend support to both possibilities, as well as a third, that there need be no difference in C-value between them. In the genus Hordeum, for example, the 2x C-values show no significant difference between annual and perennial taxa, though it should be noted that there is little variation in 2x C-value amongst the taxa listed. On the other hand the diploid ($2n=14$) species of Lathyrus show a definite tendency for the perennials to have a higher C-value than the annuals. But in the $2n=8$ species of Crepis the annuals had the highest and lowest C-values with the perennials having the medium values. Obviously the situation is more complex than a simple dichotomy between annual and perennial, since the breeding system, which is not listed, will also have an effect.

The finding of tetraploids with less DNA than related diploids is not an uncommon one, as Seal (1983) found in Pestuca and Jones and Rees (1968) in Allium. In the case of Pestuca there was also a decreasing 2x value with increasing ploidy level. One of the explanations put forward for this was that during the course of evolution the diploids have gained DNA or the polyploids have lost some.

3.4.5₁ TANNINS

It had long been my experience with Reynoutria that good staining could not be achieved with Feulgen. Thus, at a time when all mitotic cytology at Leicester was carried out with Feulgen, I was alone in the use of acid hydrolysis and aceto-orcein. Moreover, good staining could be achieved only with certain batches of stain.

Work on the failure of Feulgen staining in the 1940s and 1960s seems to have been concerned with the so-called Feulgen negative algae such as Oscillatoria. Ishida (1961), working on the effects of various compounds on the in vitro Feulgen staining of DNA, came to the following conclusions. Firstly, the amino acid l-tryptophane gave a 17% reduction in Feulgen staining intensity. Secondly, various proteins extracted from algae greatly reduced the colour intensity of Feulgen staining of calf thymus DNA. Thirdly, the addition of tannins to solutions of DNA did not reduce the staining of the DNA, but, since the absorption peak for tannin overlapped with that of Feulgen and tannin itself gave a positive results with Feulgen, increased Feulgen intensity was obtained with such mixtures.

Leeman and Ruch (1983) found with their fluorescent studies on Rumex acetosa that the type of fixation had a profound effect on the amount of fluorescence achieved. In their graph (reproduced as Figure 3.13) they showed that the UV absorbance peak for the unstained nuclei of R. acetosa fixed in 3:1 was 275nm instead of 260nm, and that there was more

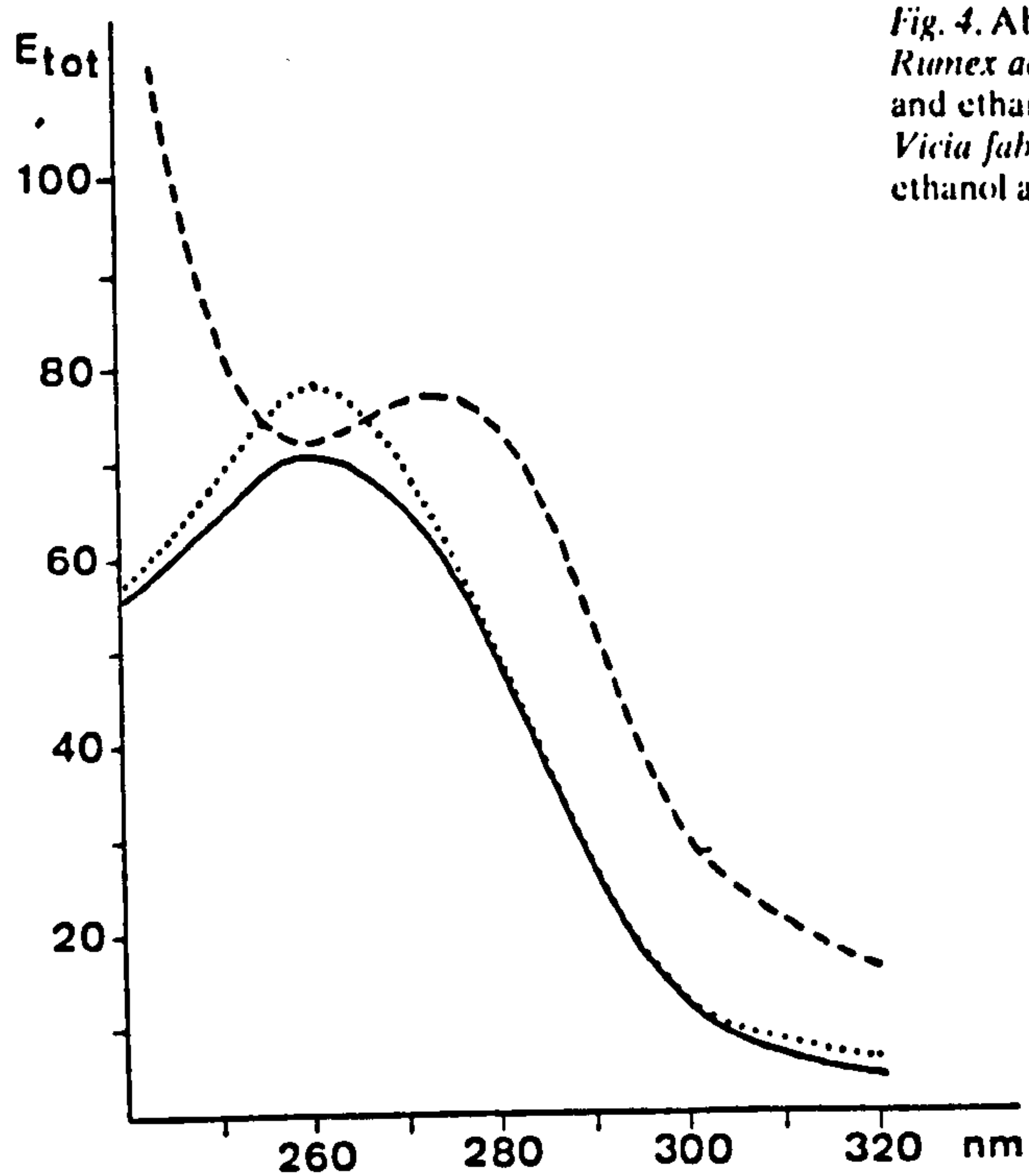


Fig. 4. Absorption spectra of cell nuclei of *Rumex acetosa* fixed in formalin (—) and ethanol-acetic acid (---), and of *Vicia faba* (....., $E_{tot} \times 0.3$), fixed in ethanol acetic acid.

FIGURE 3.13 EFFECT OF FIXATION SOLUTION ON THE U.V.

ABSORPTION SPECTRUM OF RUMEX ACETOSA DNA.

Reproduced from Leeman and Ruch 1983

deviation in the measurements. Root tips fixed in 4% neutral formalin gave more normal UV absorption spectra, as did roots of Vicia faba fixed in ethanol. From their results they proposed that Rumex "probably produces a UV absorbing substance which interferes with DNA staining."

Greilhuber (1986), in an examination of Pinus species fixed in 3:1 or 4% formalin, found that the 3:1-fixed material gave a much lower staining density and hence DNA C-value than formalin-fixed material. This, he suggested was caused by the presence of tannins in the vacuome of the Pinus meristematic cells. In Pinus mugo formalin fixation gave a C-value nearly 2.5 times greater than equivalent material fixed in 3:1. Greilhuber considered that the tannins were soluble in 3:1 but were immobilized by formaldehyde. With 3:1 fixation the tannins escape from their vacuoles and tan the cellular structures including the chromatin, thus physically interfering with the Feulgen staining. Reynoutria cells fixed with formalin (Plate 3.8) reveal numerous heavily pigmented inclusions when stained with Giemsa or orcein, but in 3:1 fixed preparations the cytoplasm is entirely clear. At first sight Greilhuber's work might appear to contradict that of Ishida (1961). However, Ishida was working on pure DNA in vitro, and since the action of tannins is probably on the protein component of chromatin these results are not necessarily conflicting.

Greilhuber (1986) stated that the tannins found in Pinus are probably condensed tannins. Newbury and Possingham (1977), working on Vitis, stated that condensed tannins are

proanthocynadins which turn red on heating with dilute HCl to produce anthocyanidins. The fact that the root tips of my taxa are generally coloured pink after the HCl hydrolysis (but before Feulgen staining), and their positive deep red colouration with the Vanillin/H₂SO₄ test, suggest that they too contain condensed tannins in their vacuoles.

The presence of large amounts of vacuolar tannins is a serious problem for a cytologist. Although the use of formaldehyde fixation keeps this in check enough for DNA estimations by Feulgen microdensitometry, it seems that formalin fixation is not the entire answer. Leeman and Ruch (1983) mention that even with formalin fixation the UV absorbing substance is still capable of affecting the fluorescence staining abilities of cells, and conclude: "since for many staining reactions, including DAPI and CMA, alcoholic fixatives are usually recommended, and since good chromosome spreads cannot be obtained with formalin, the use of Rumex acetosa as a model object for eu- and heterochromatin is somewhat limited". The same statement must also apply to Reynoutria and Fallopia taxa.

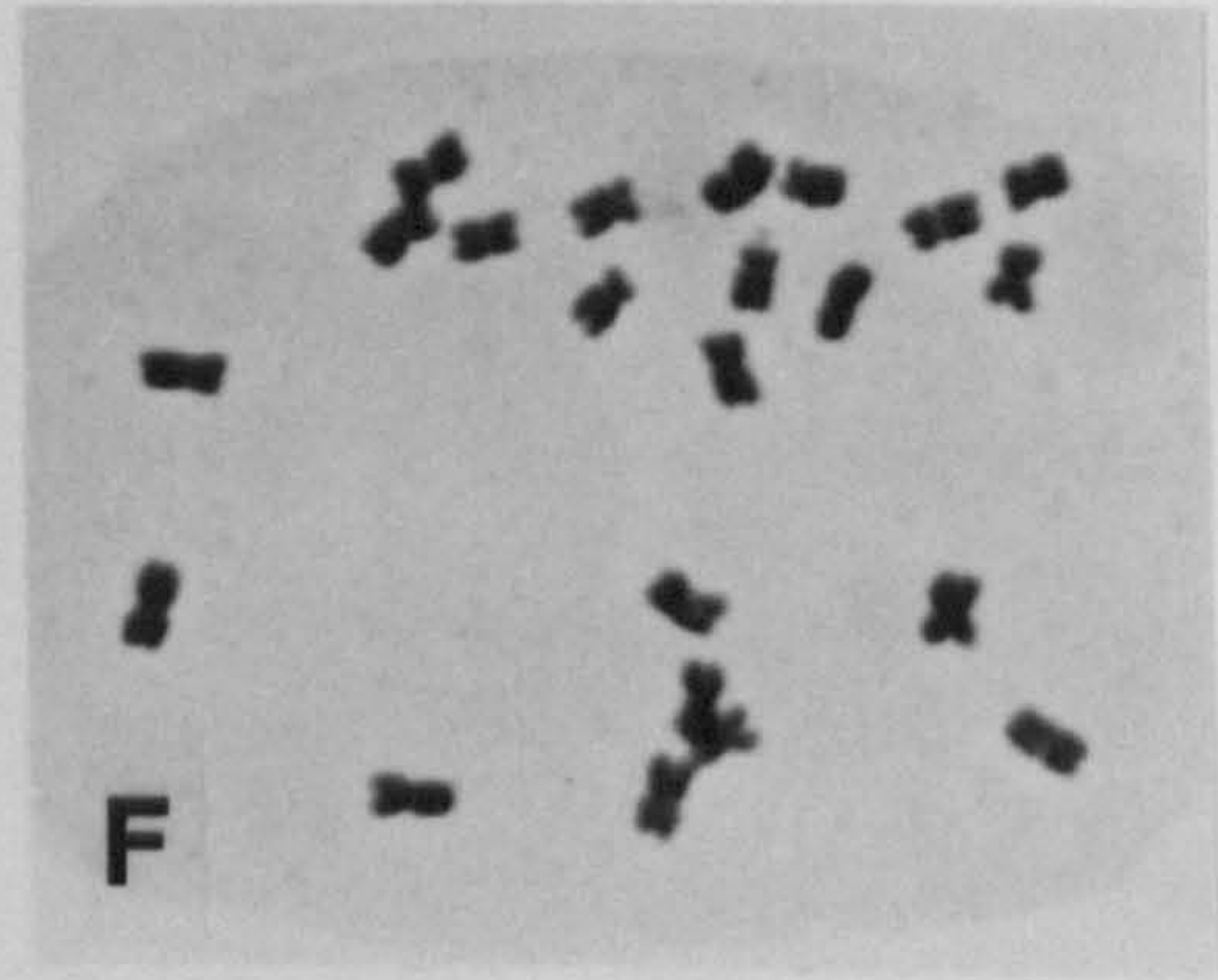
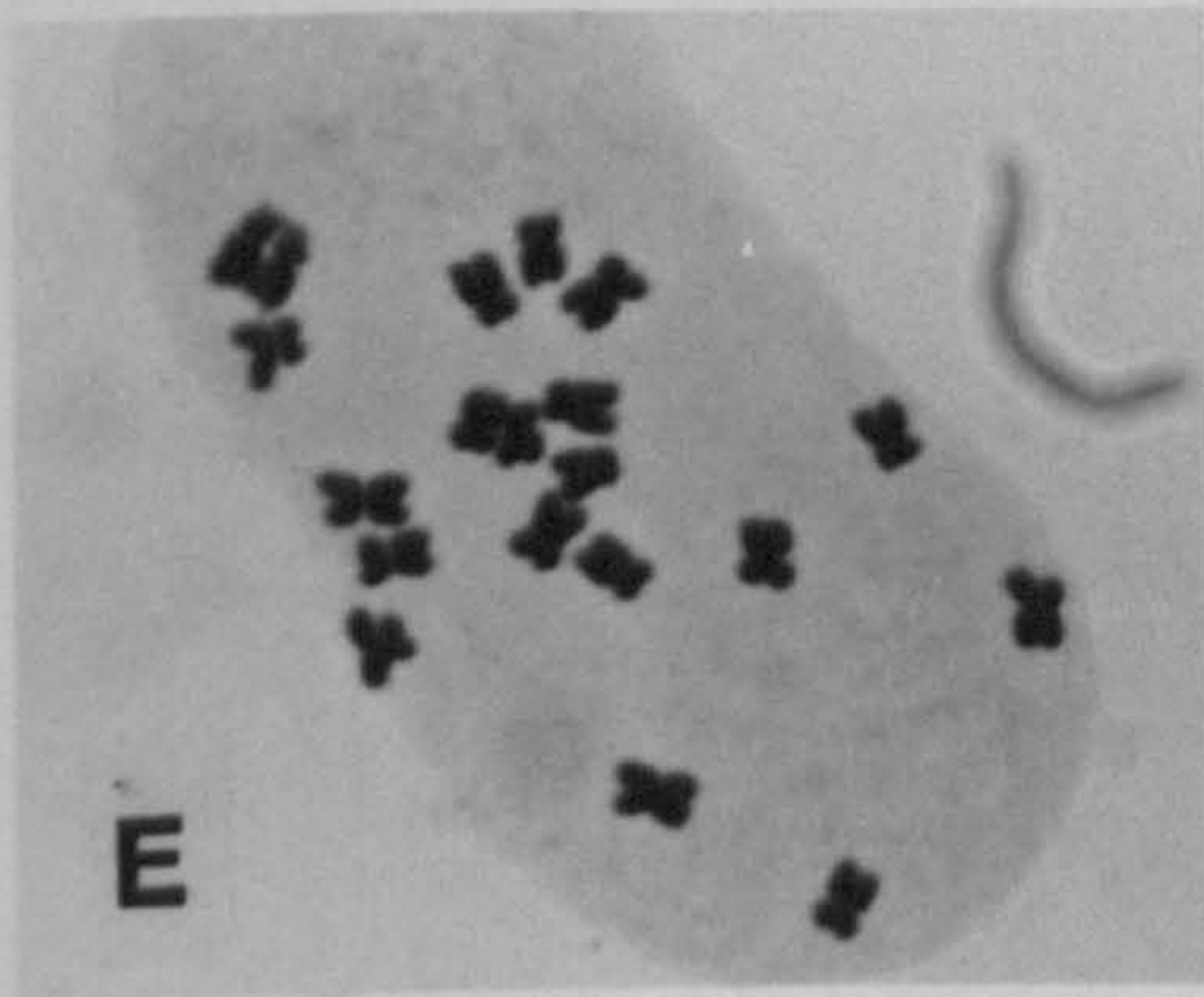
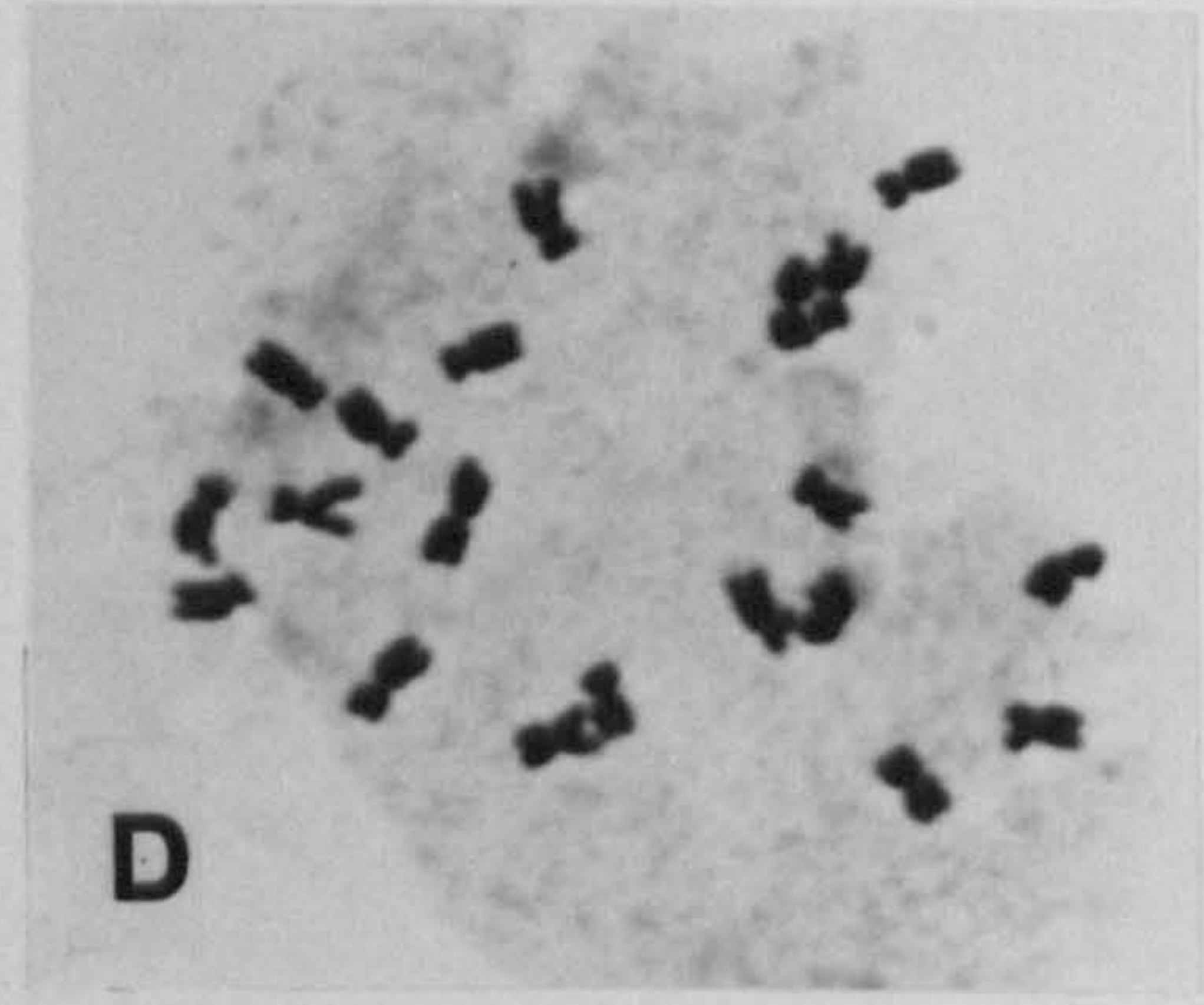
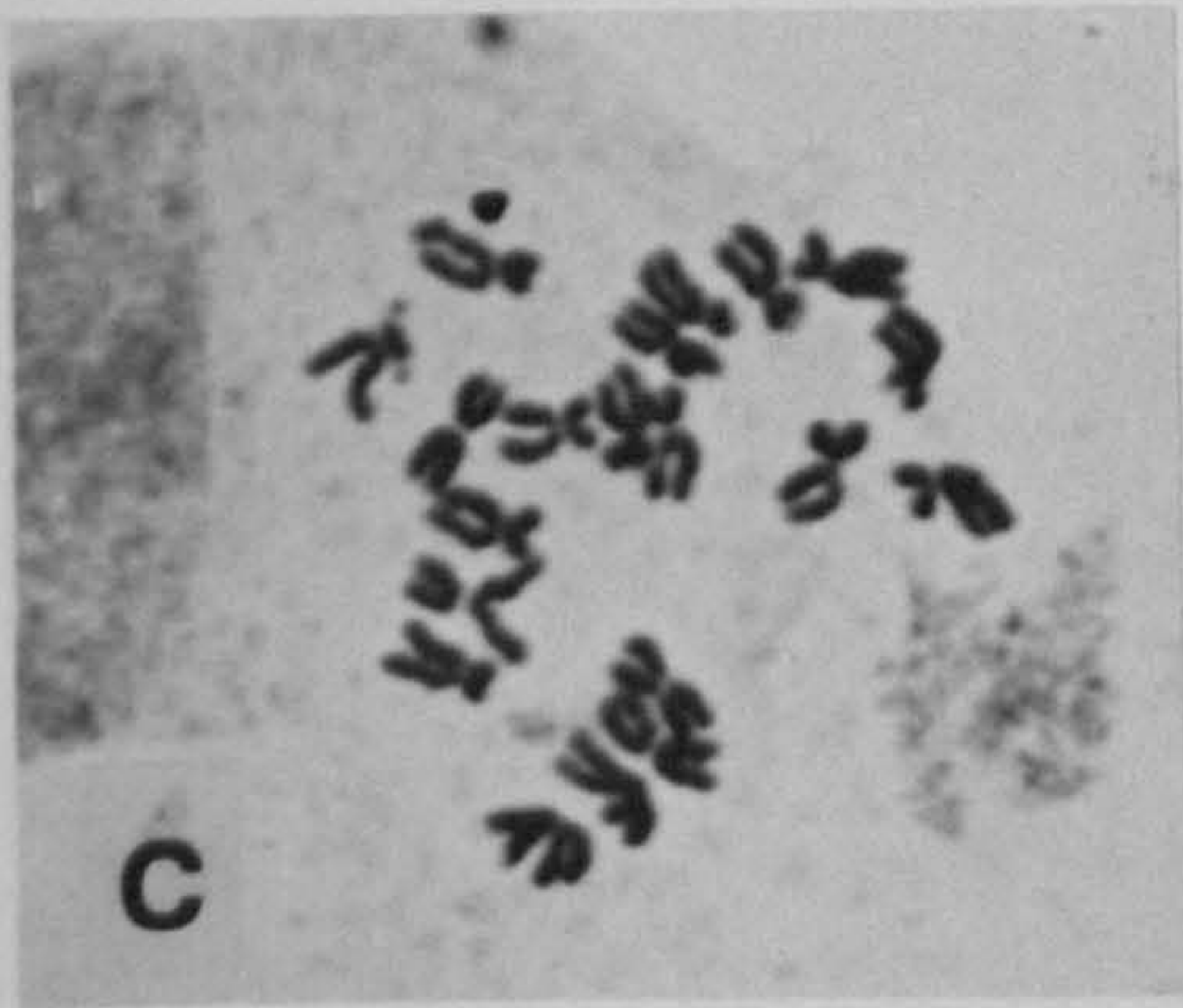
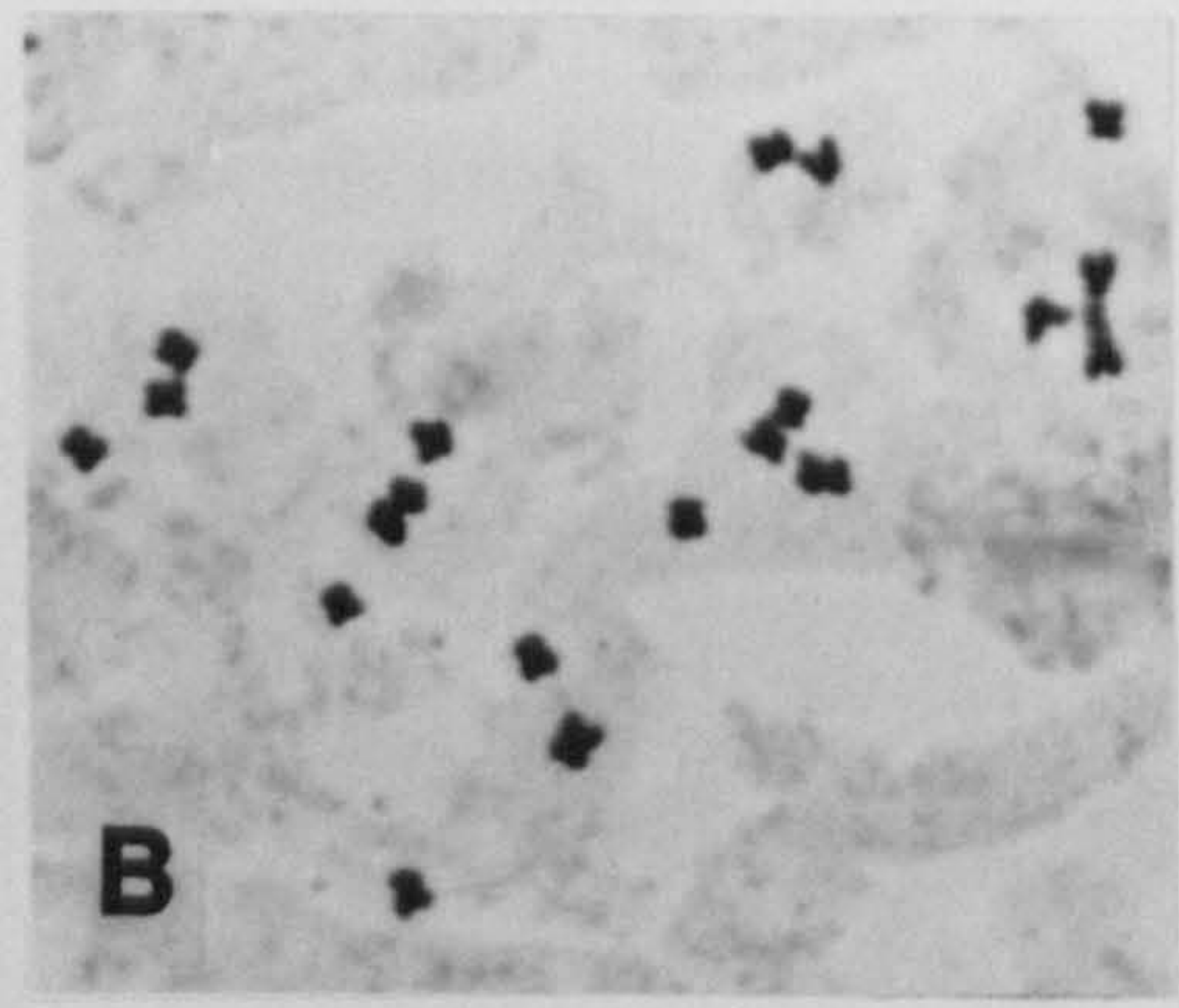
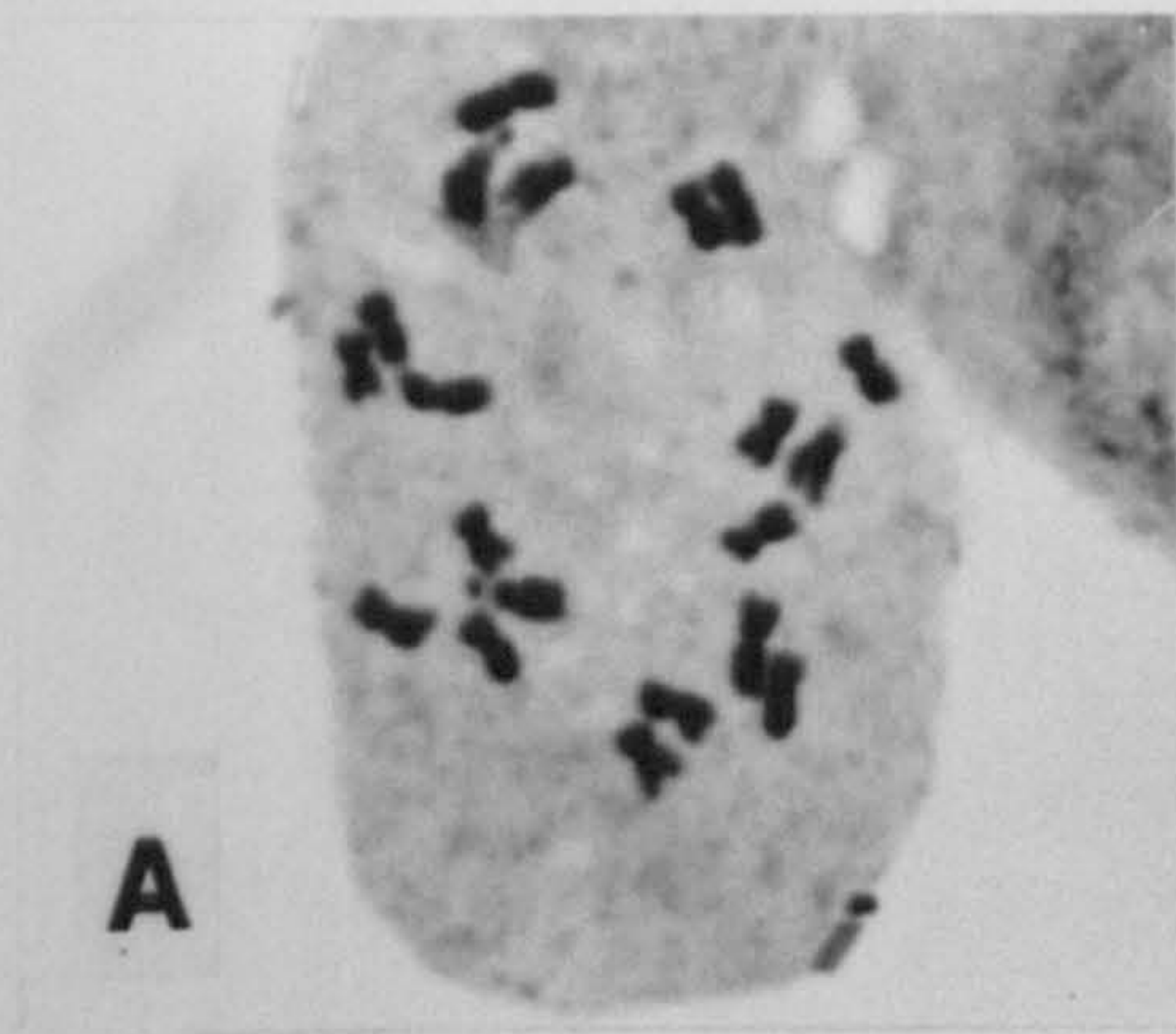


Plate 3.1 Diploid mitosis

A,B *F. scandens* var. *dumetorum*
note difference in chromosome
contraction

C,D *F. baldschuanica* P 163

E *F. baldschuanica* P175

F *F. cilinodis* P148

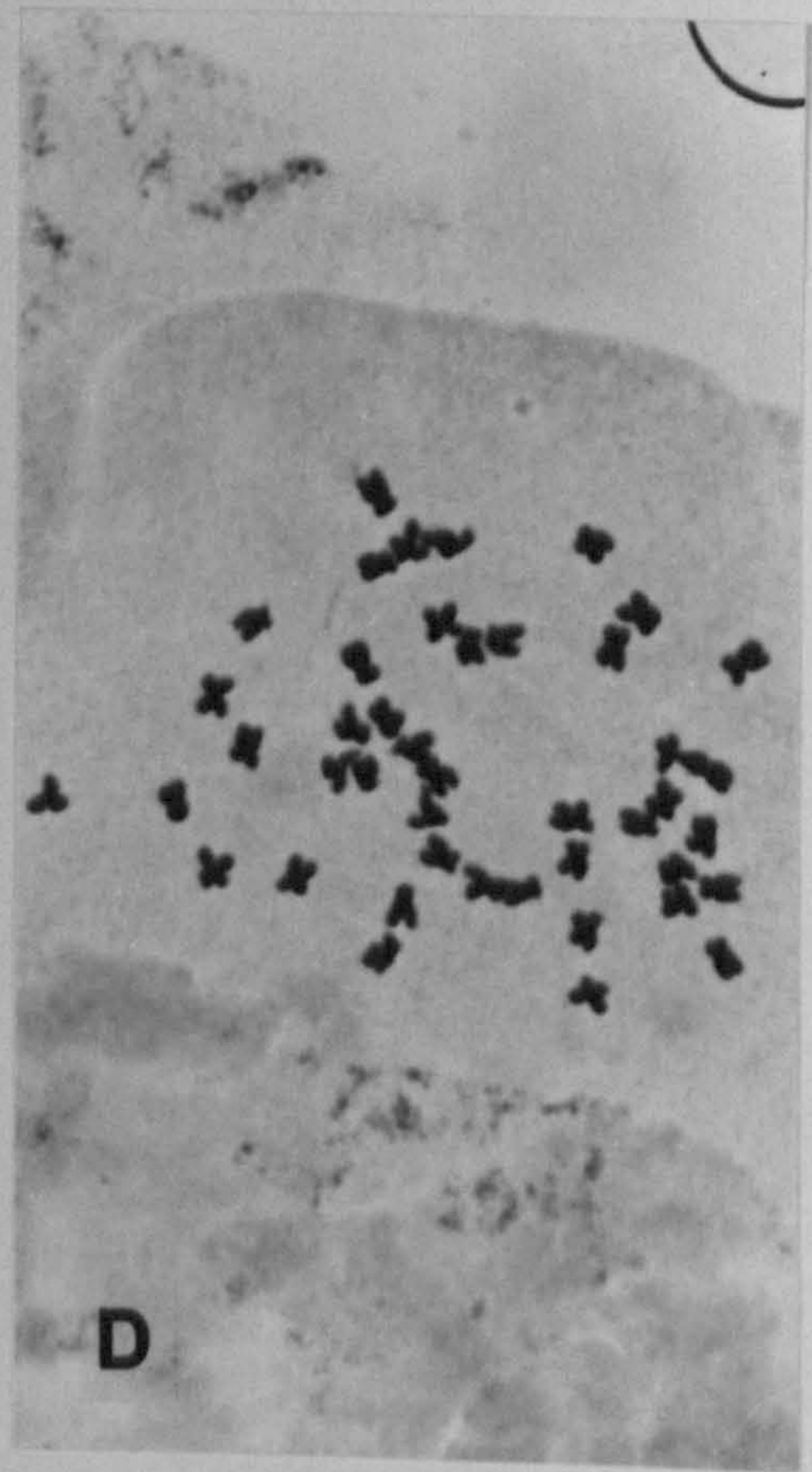
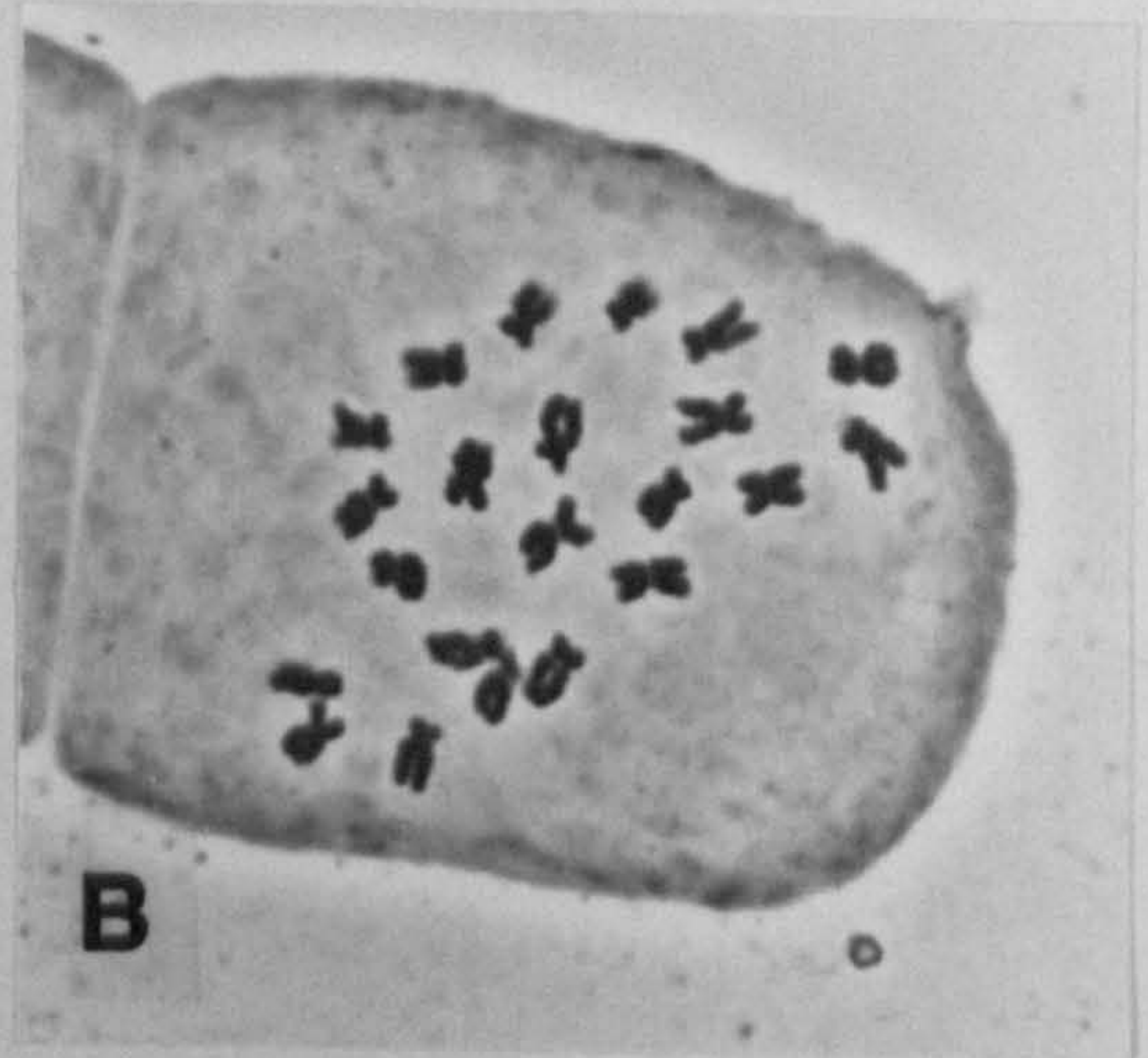
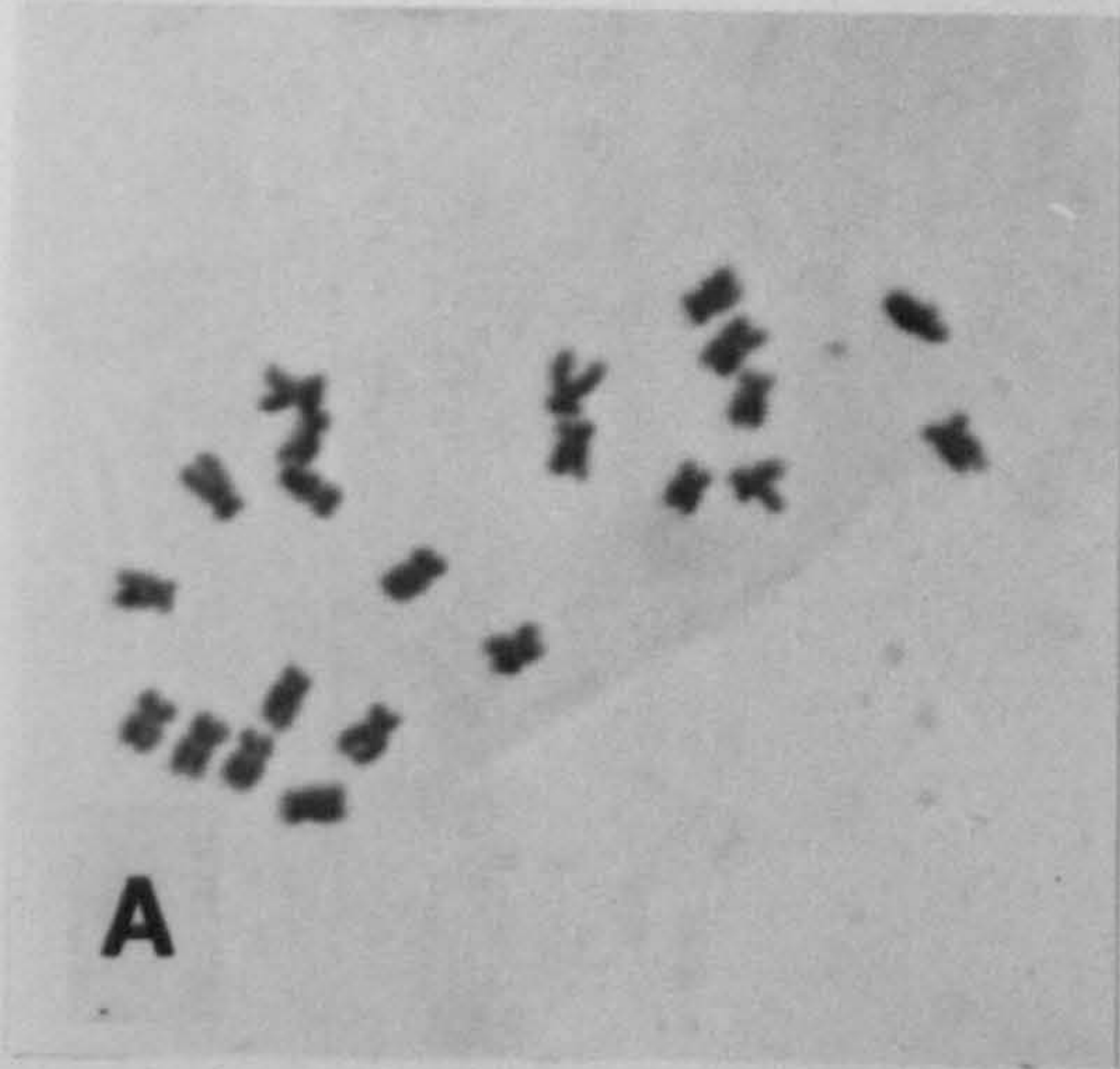


Plate 3.2 Diploid and tetraploid mitosis

A,B *F. multiflora* P162

C *F. convolvulus* P150b

D *R. japonica* P134b

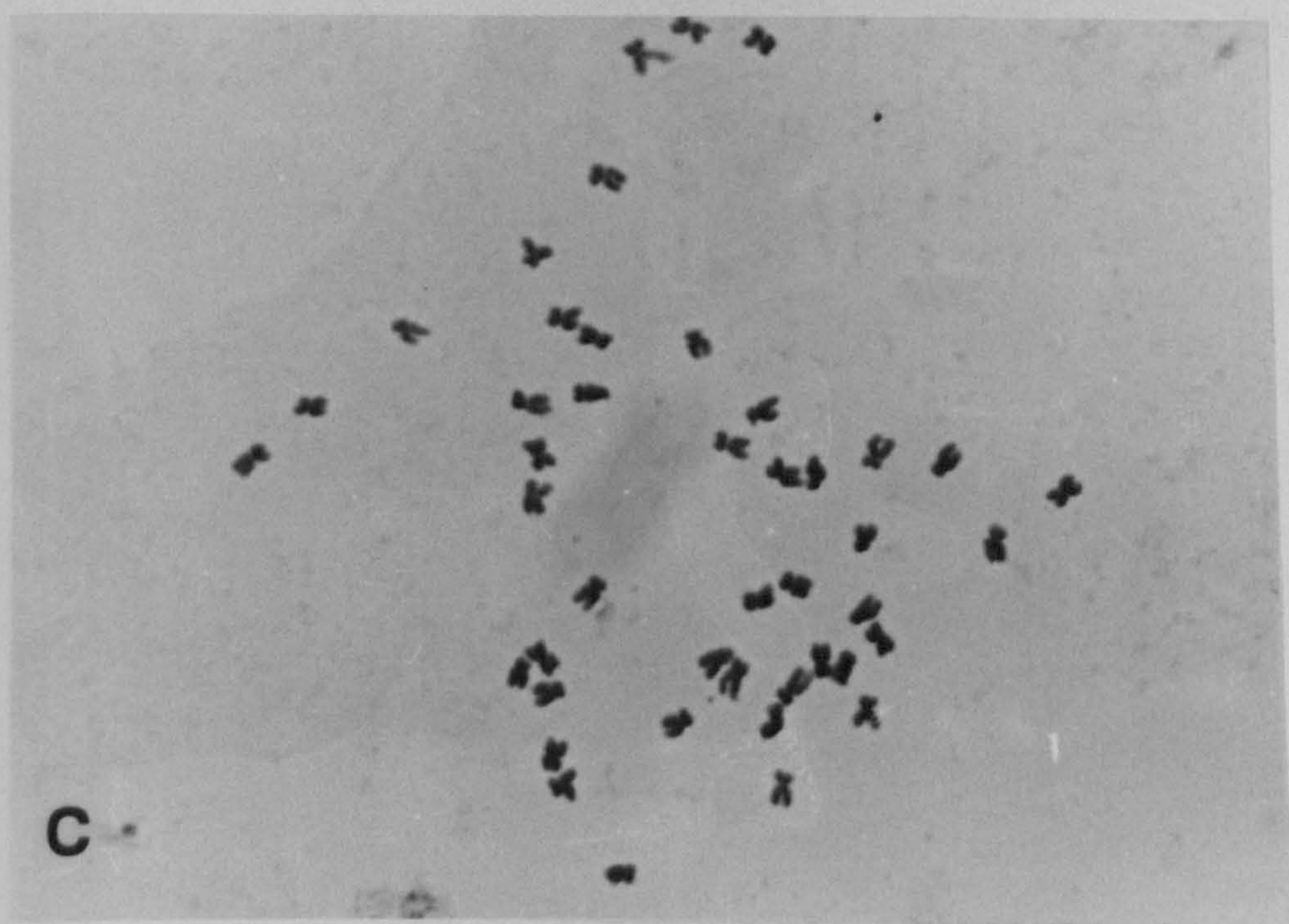
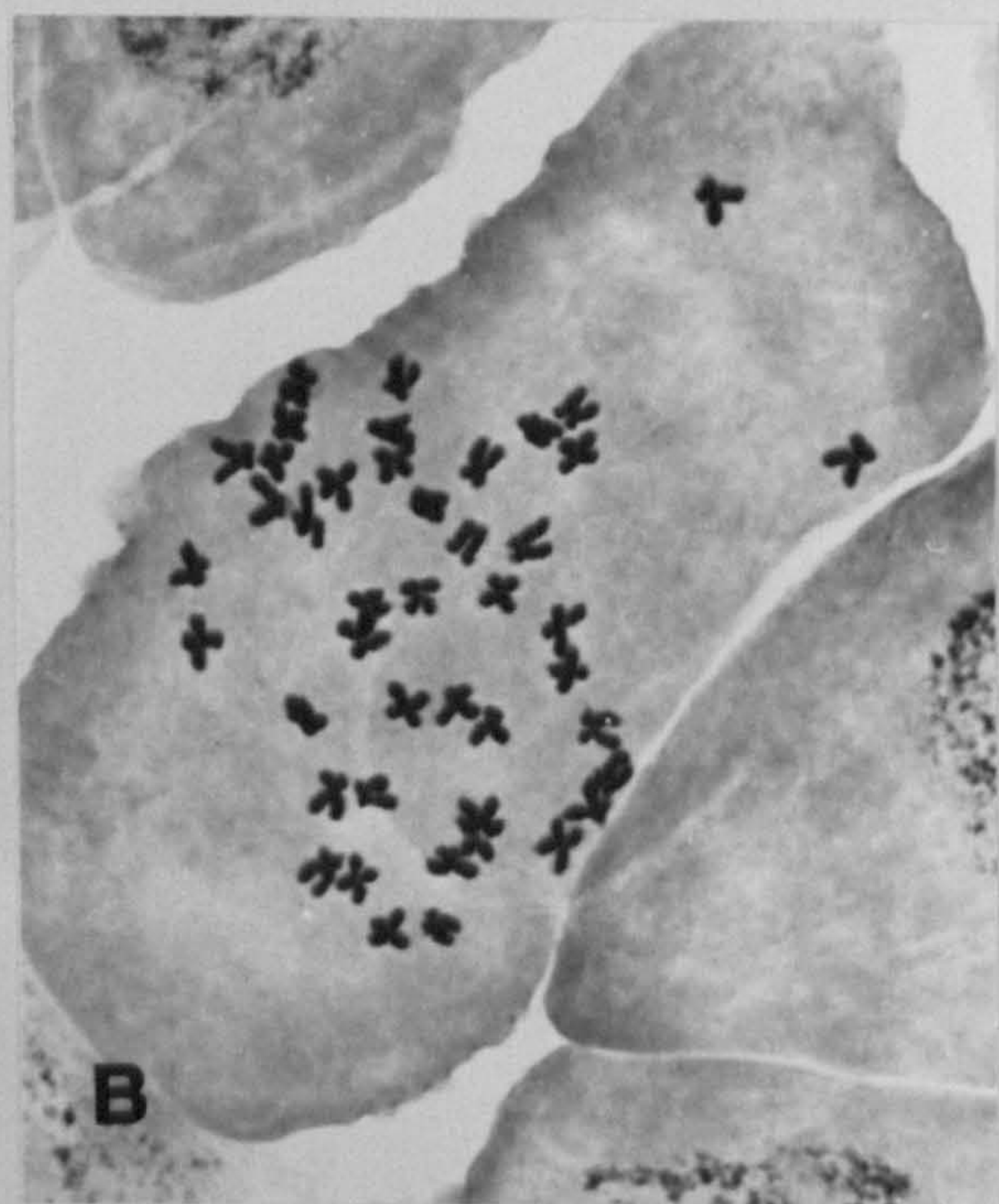
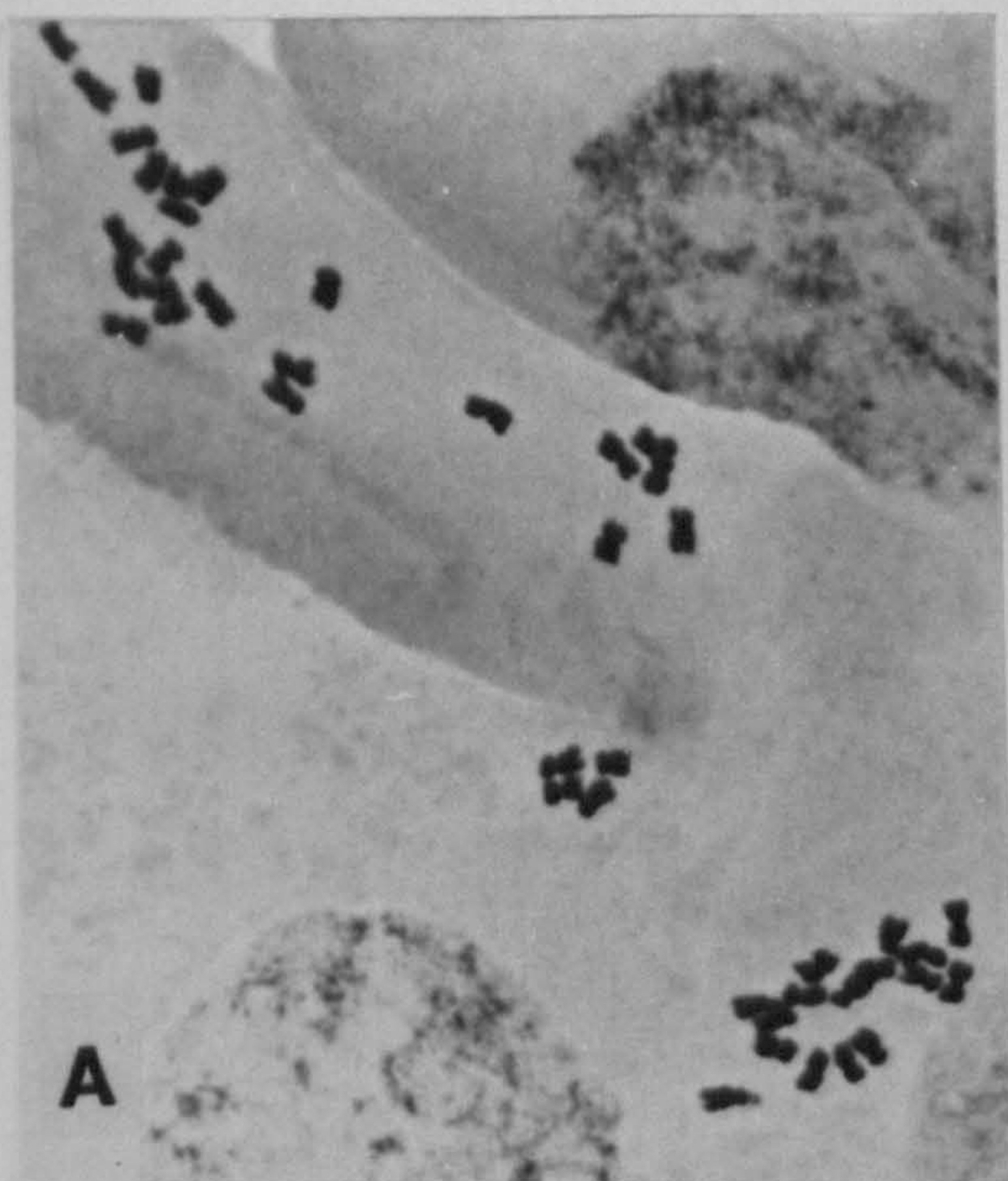


Plate 3.3 Tetraploid *Reynoutria* mitosis

- A *R. sachalinensis* P155
- B *R. japonica* P134b
- C *R. japonica* var. *compacta* P99a

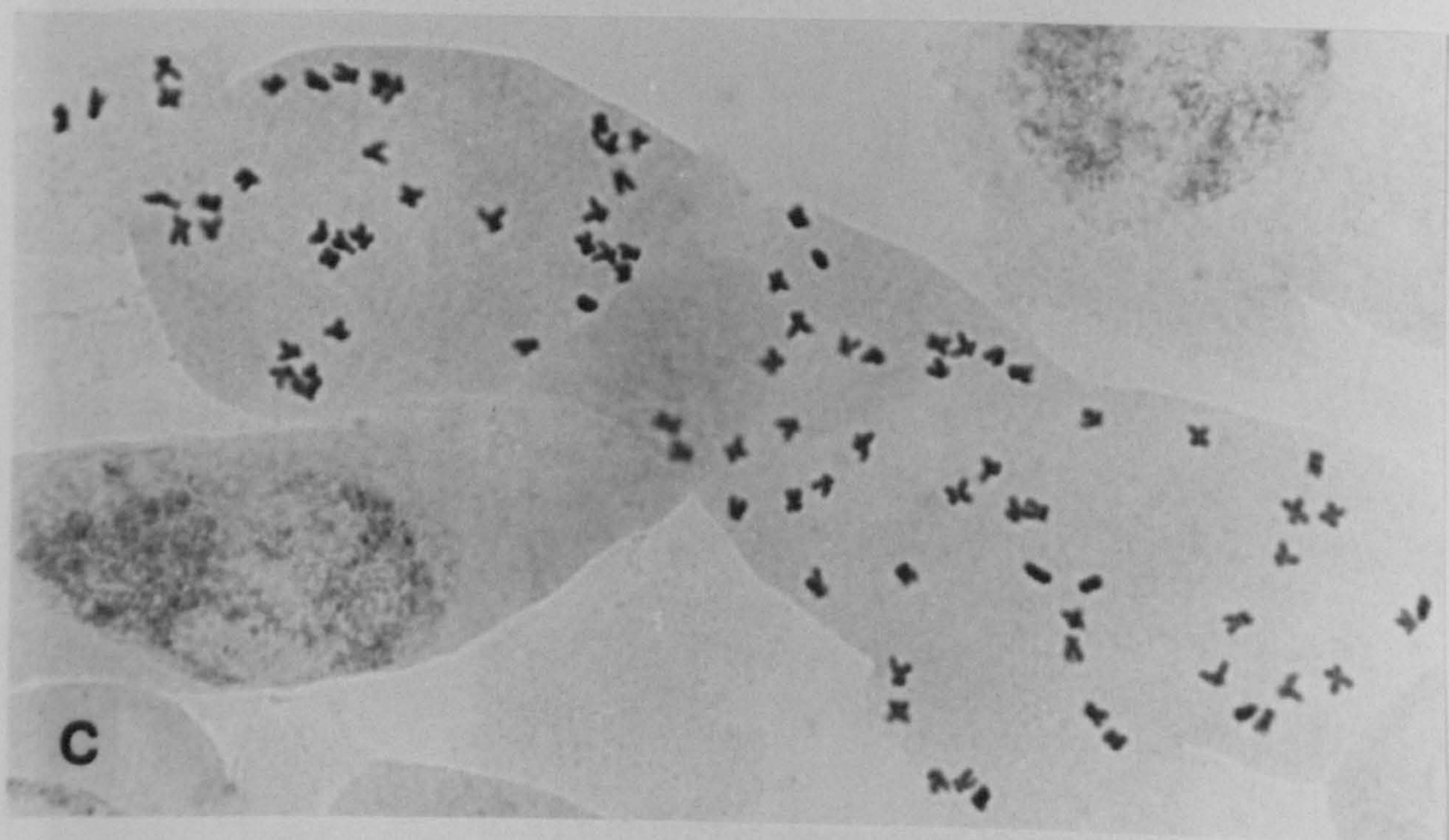
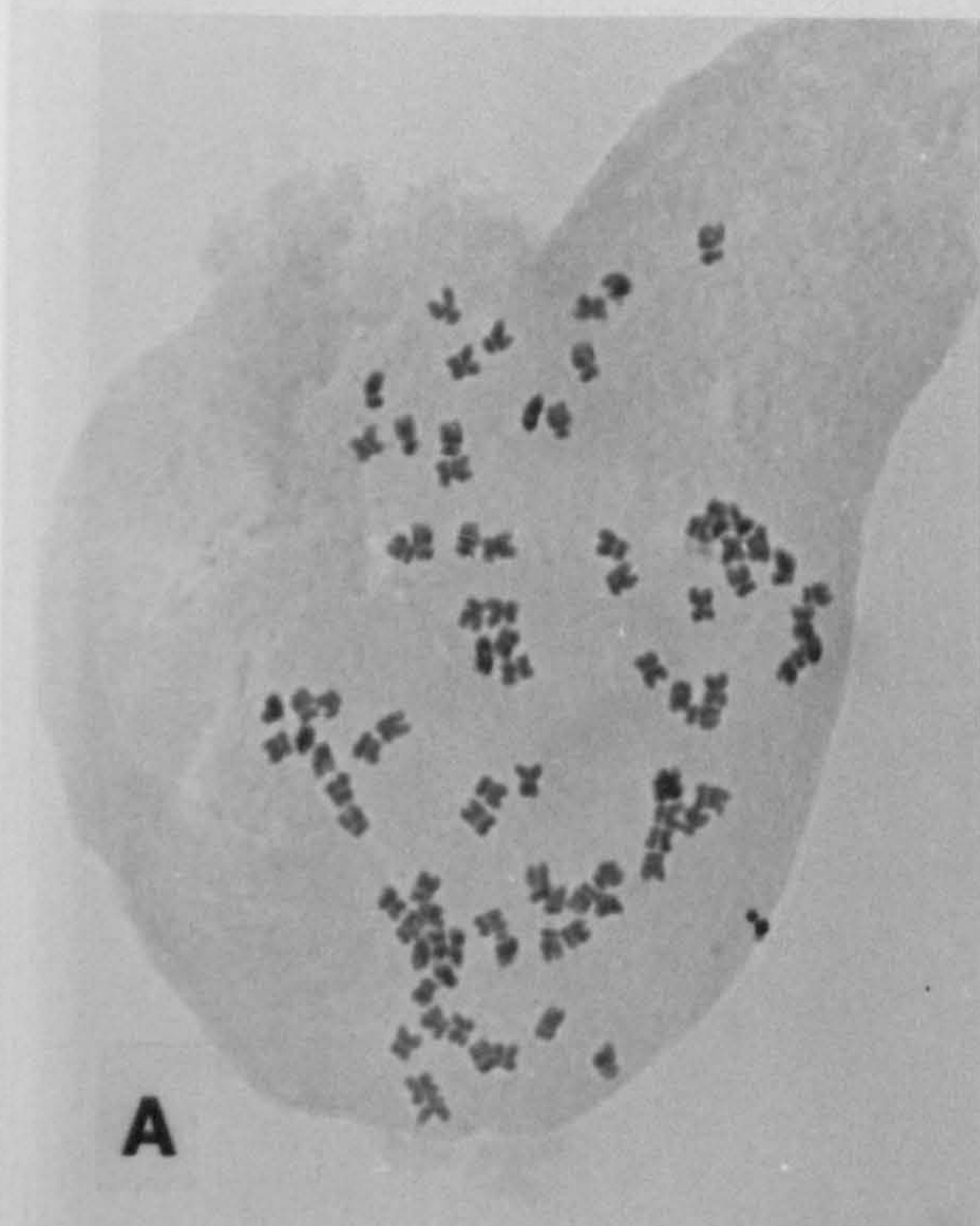


Plate 3.4 *R. japonica* mitosis

A *R. japonica* P105c

B *R. japonica* P24

C *R. japonica* P42

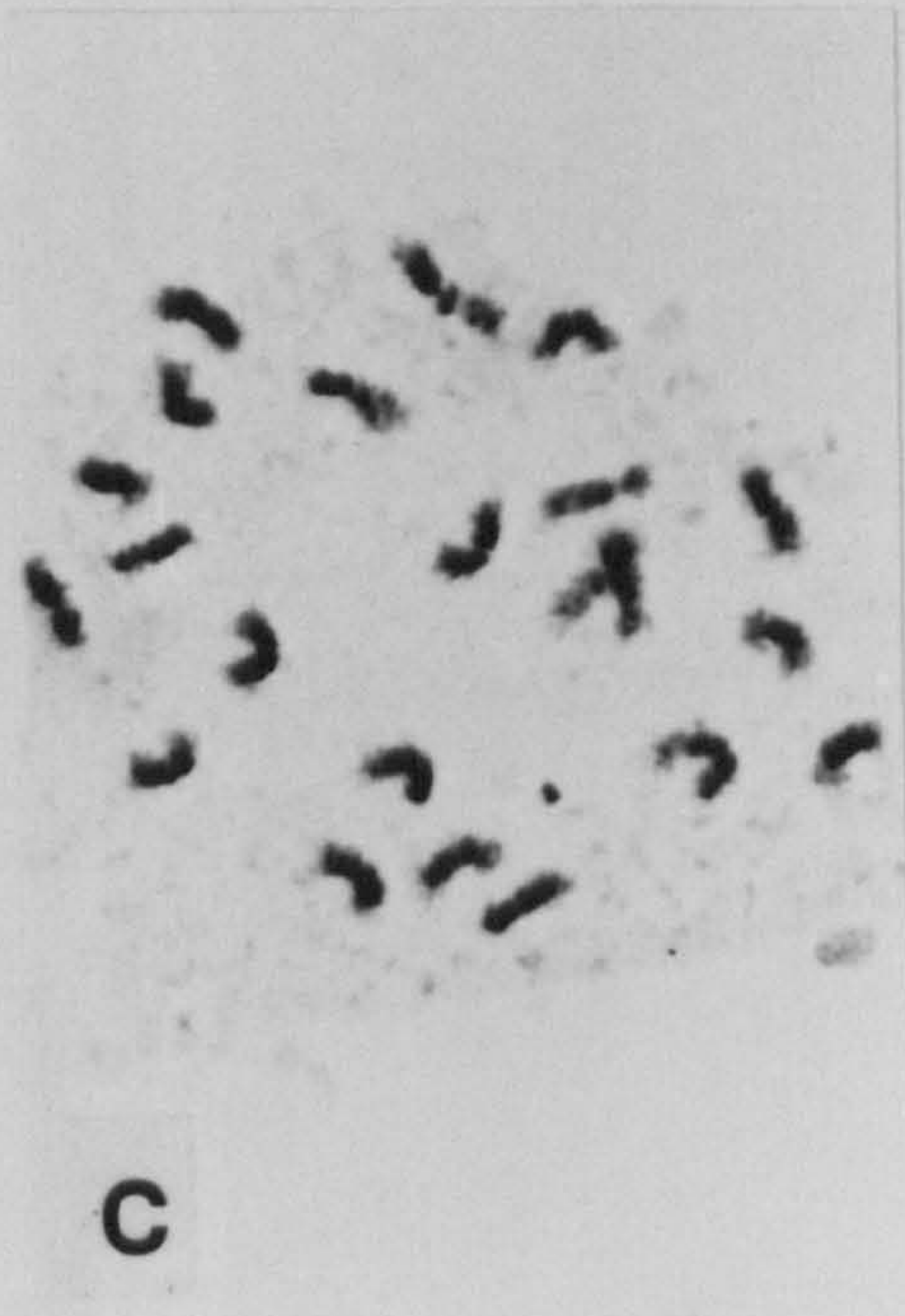


Plate 3.5 *Reynoutria* and *Fallopia* banding

A *R. sachalinensis* P137 i orcein banding

B *F. cilinodis* P156 Giemsa stained

C *F. cilinodis* P156b unstained squash, phase contrast

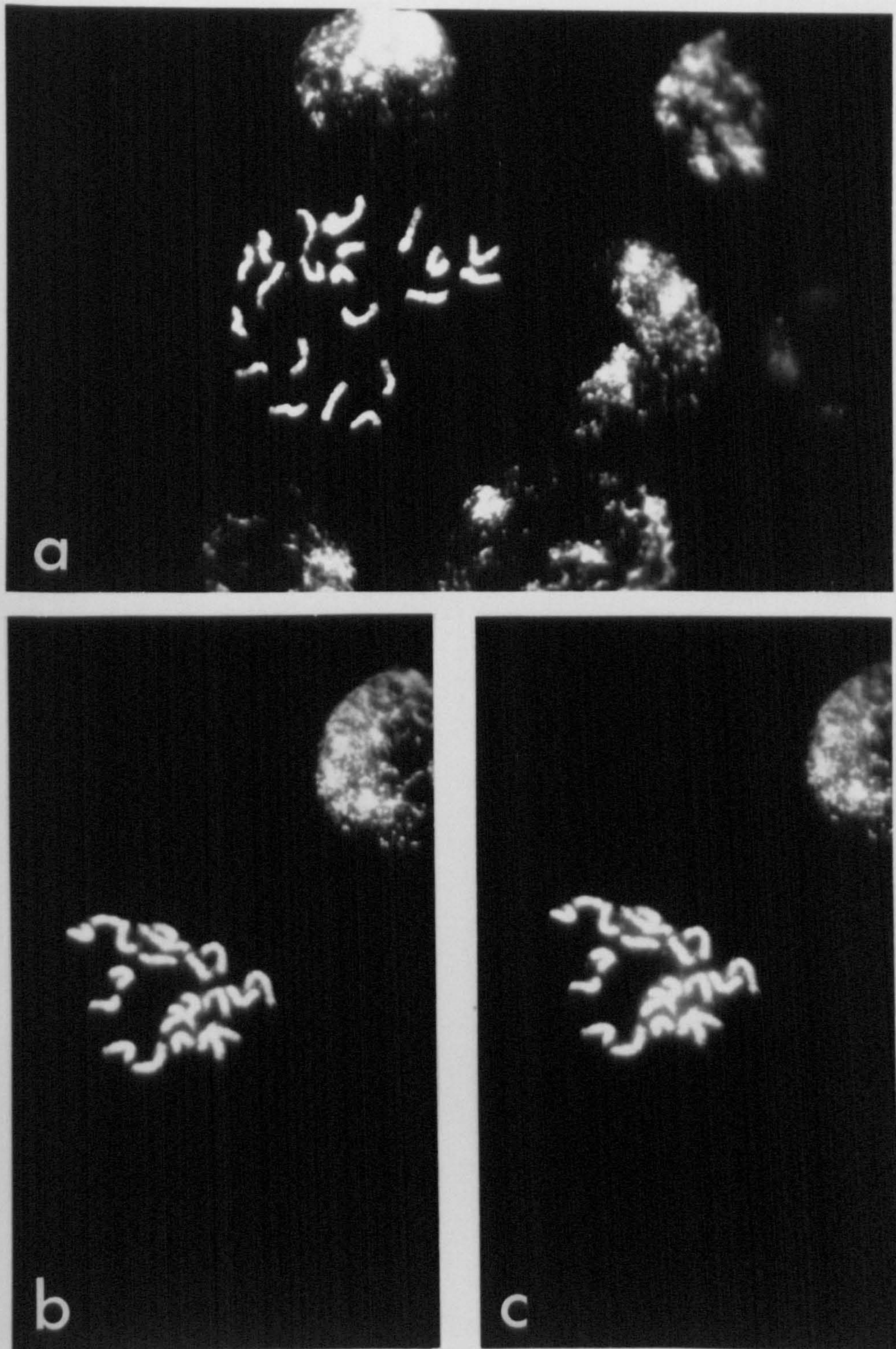


Plate 3.6 **Fluorescent staining of *F. cilinodis* P148**

A DAPI fluorescence

B DAPI fluorescence

C Preparation B viewed by chromomycin fluorescence

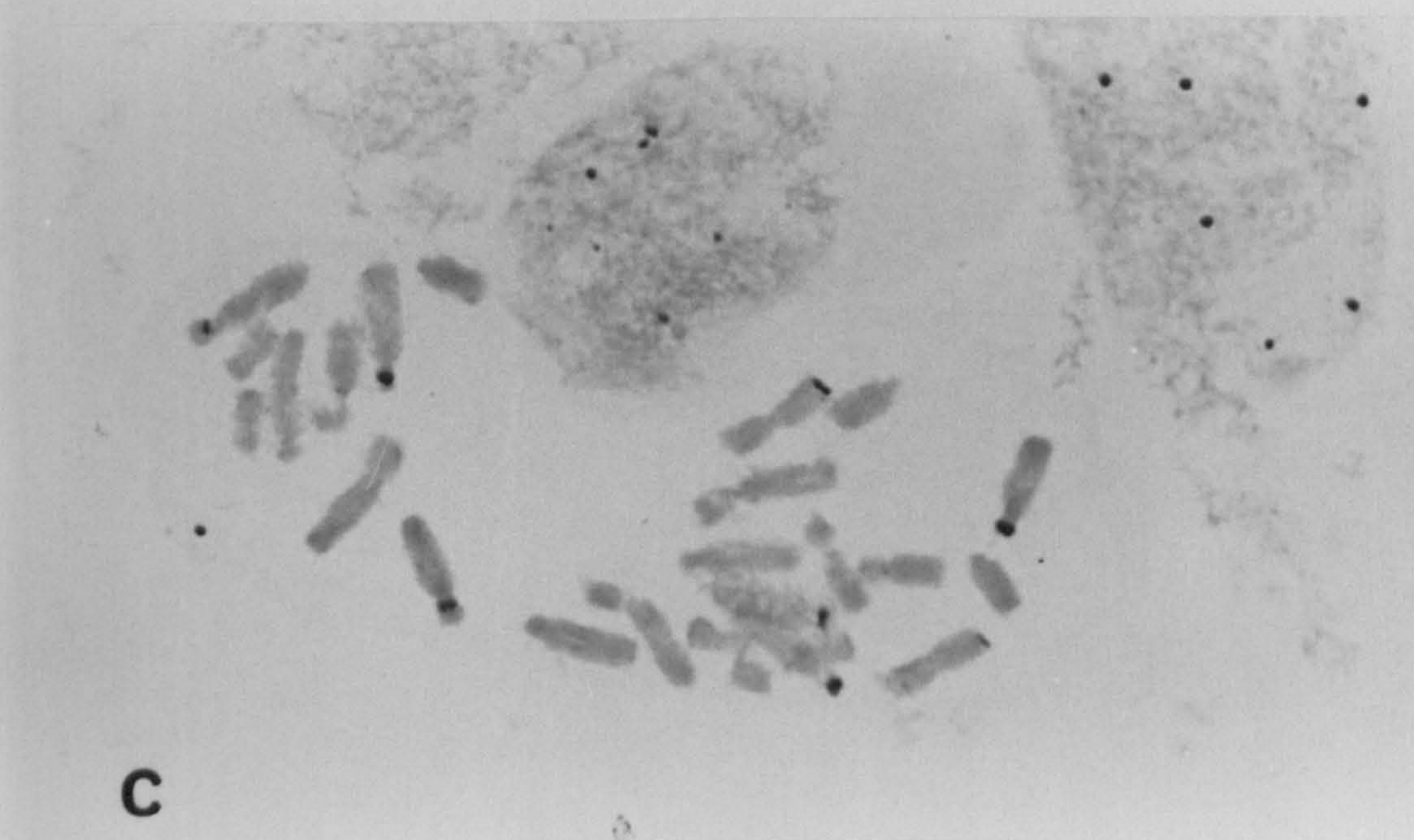
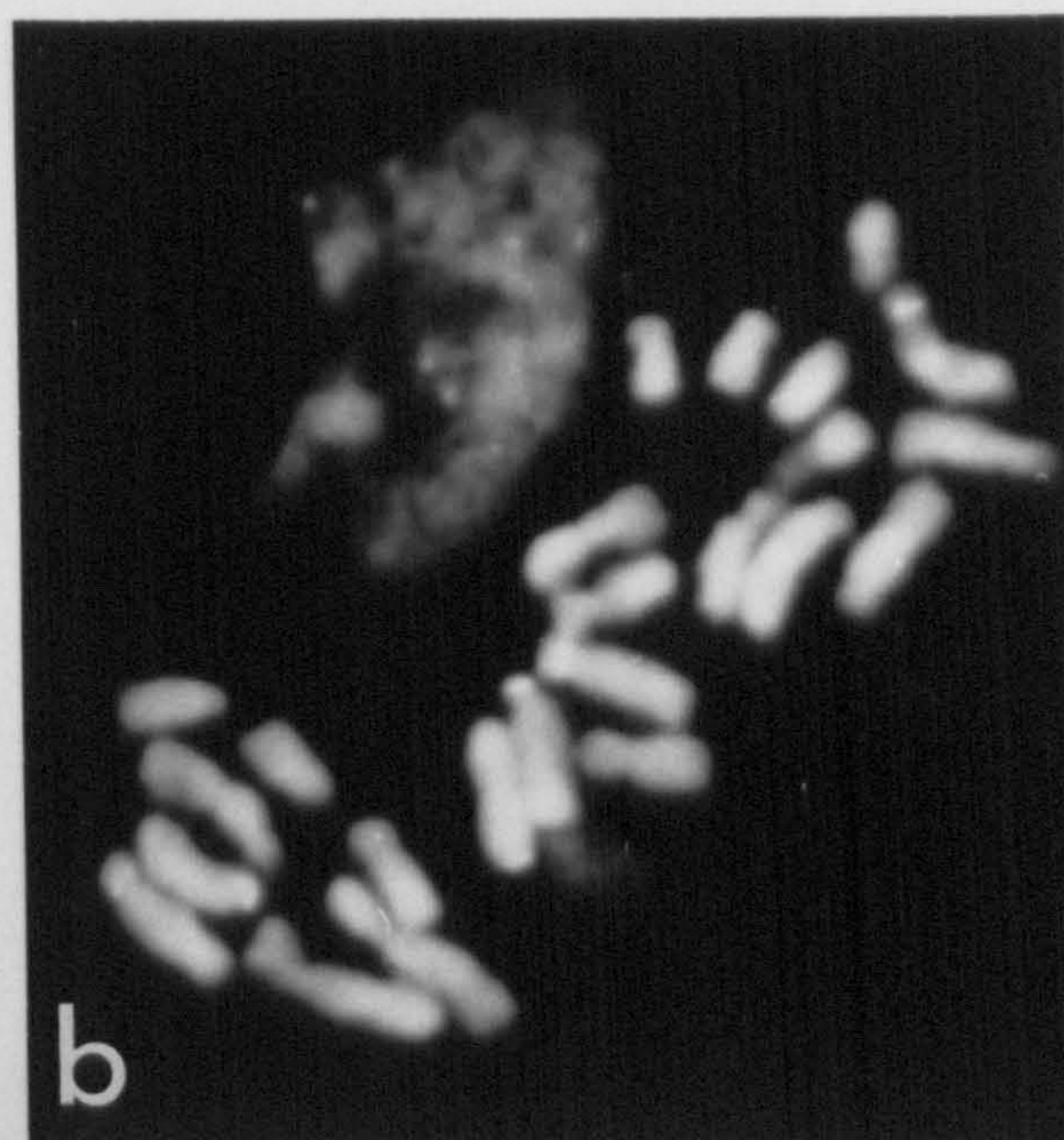
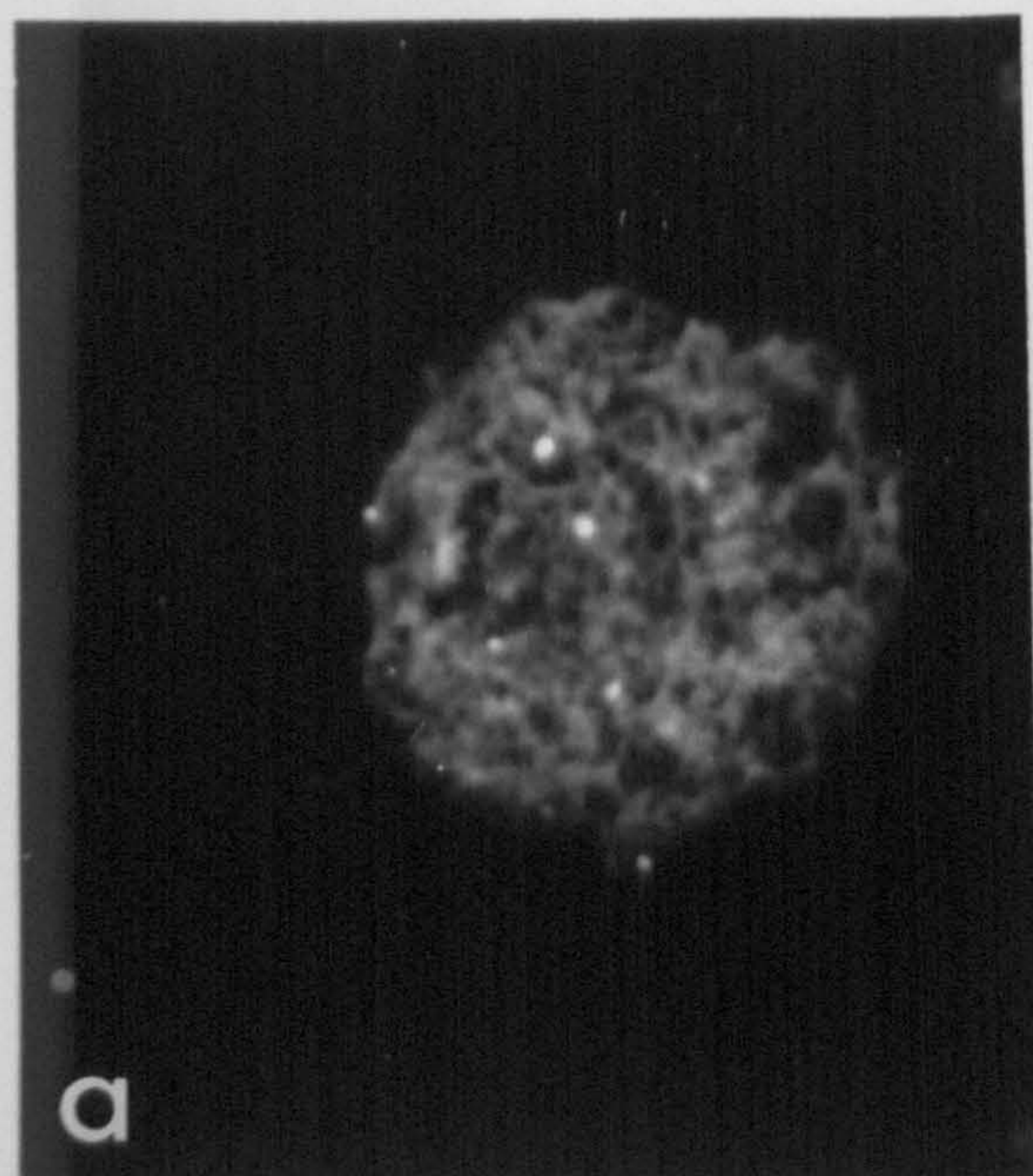


Plate 3.7 **Fluorescent and Giemsa banding of**
Hyacinthoides hispanica

- A** Chromomycin brilliant chromocentres at interphase
- B** Chromomycin brilliant material in metaphase chromosomes
- C** Giemsa banded metaphase and interphase cells

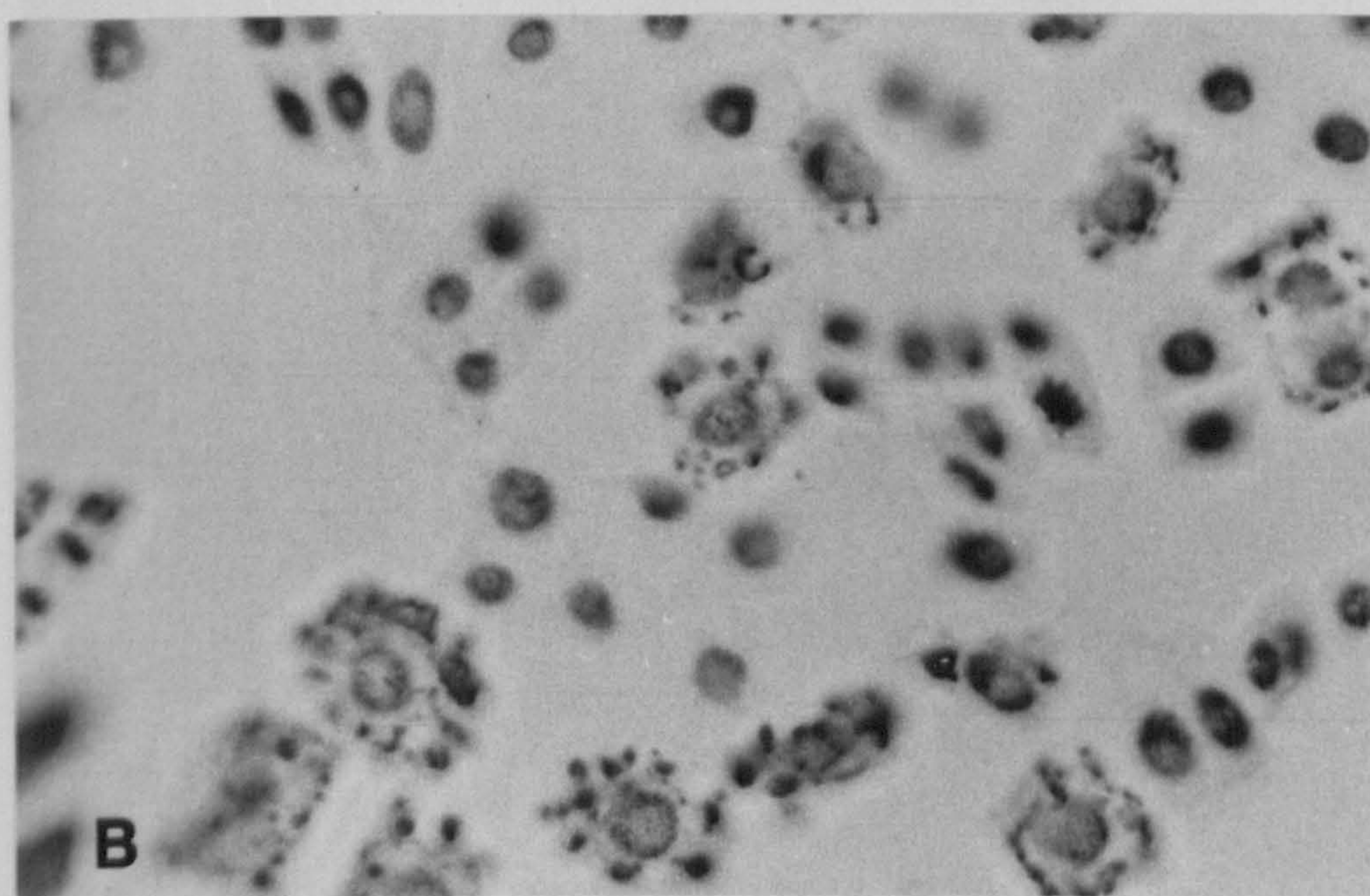
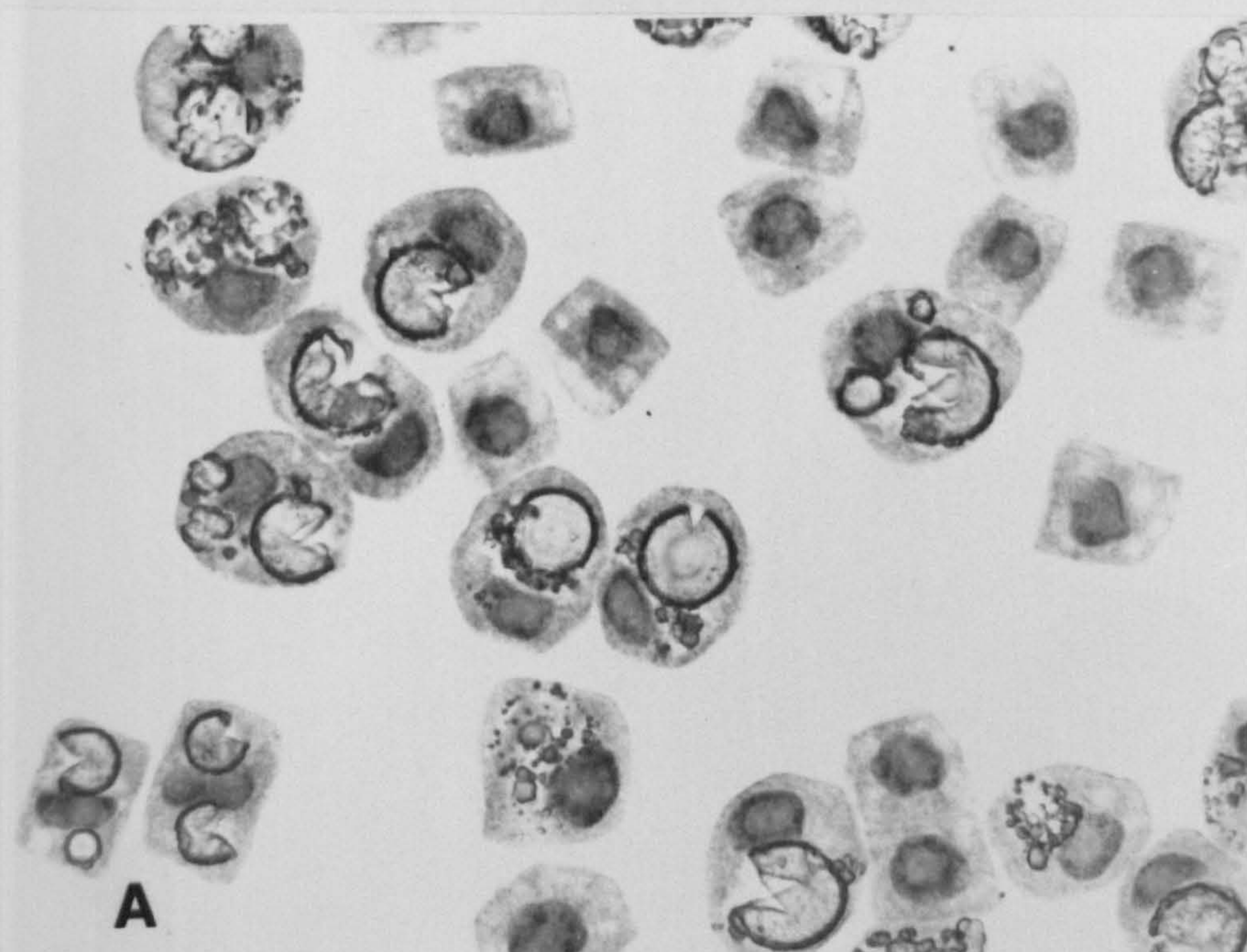


Plate 3.8 Tannin vesicles

- A** Giemsa stained preparation of P102a to show the tannin vesicles visible only in formalin fixed material.
- B** Feulgen stained squash of P162 to show tannin vesicles

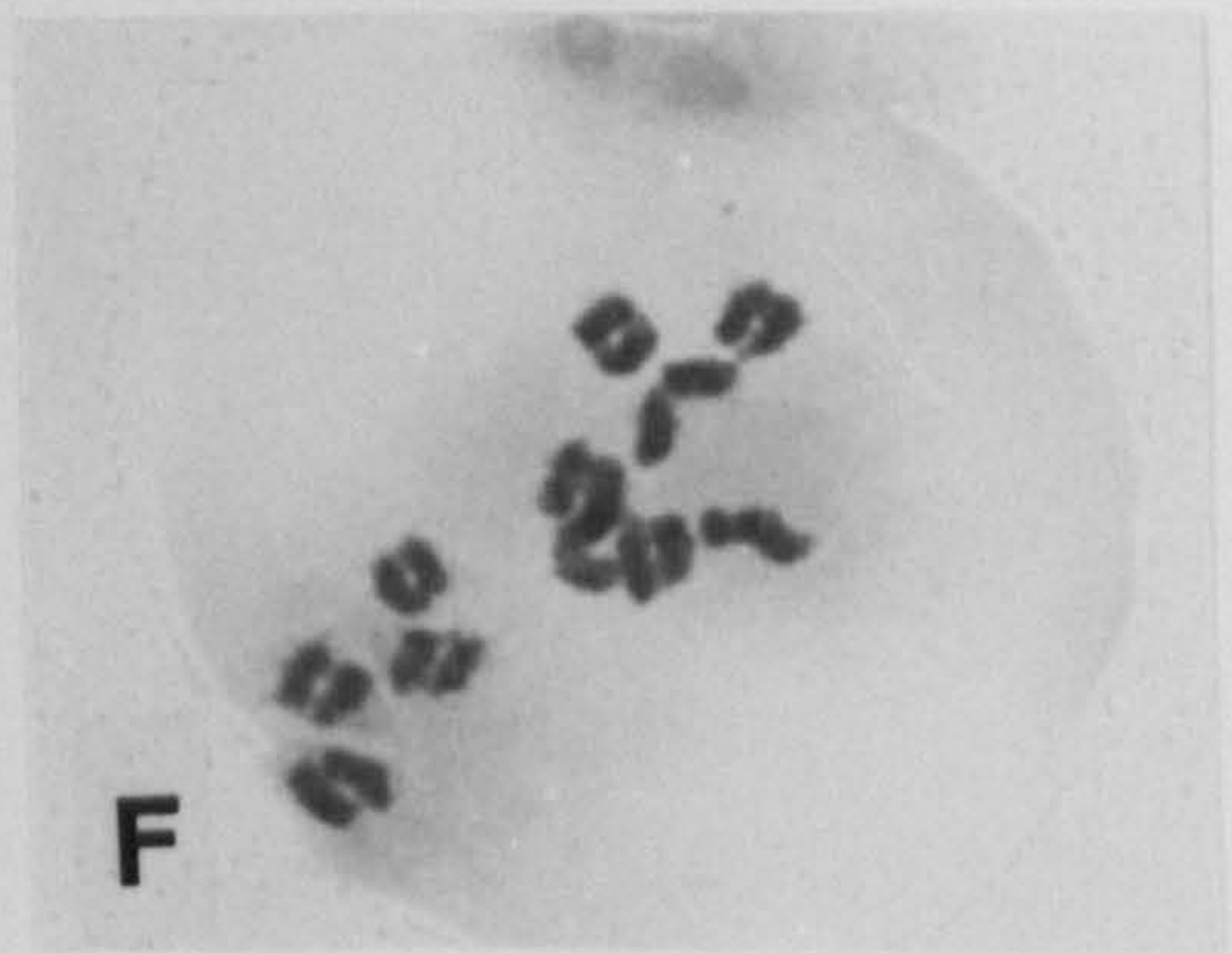
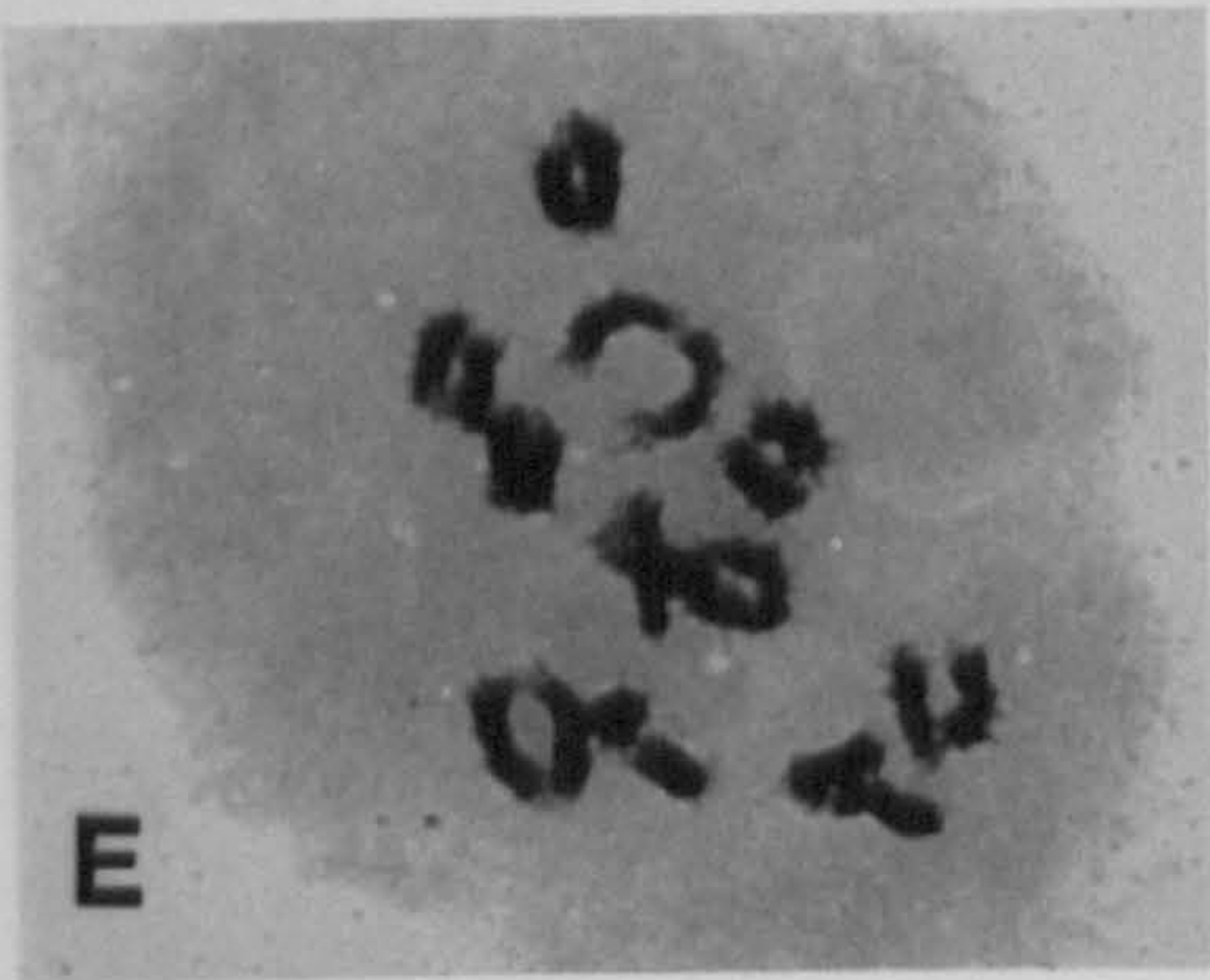
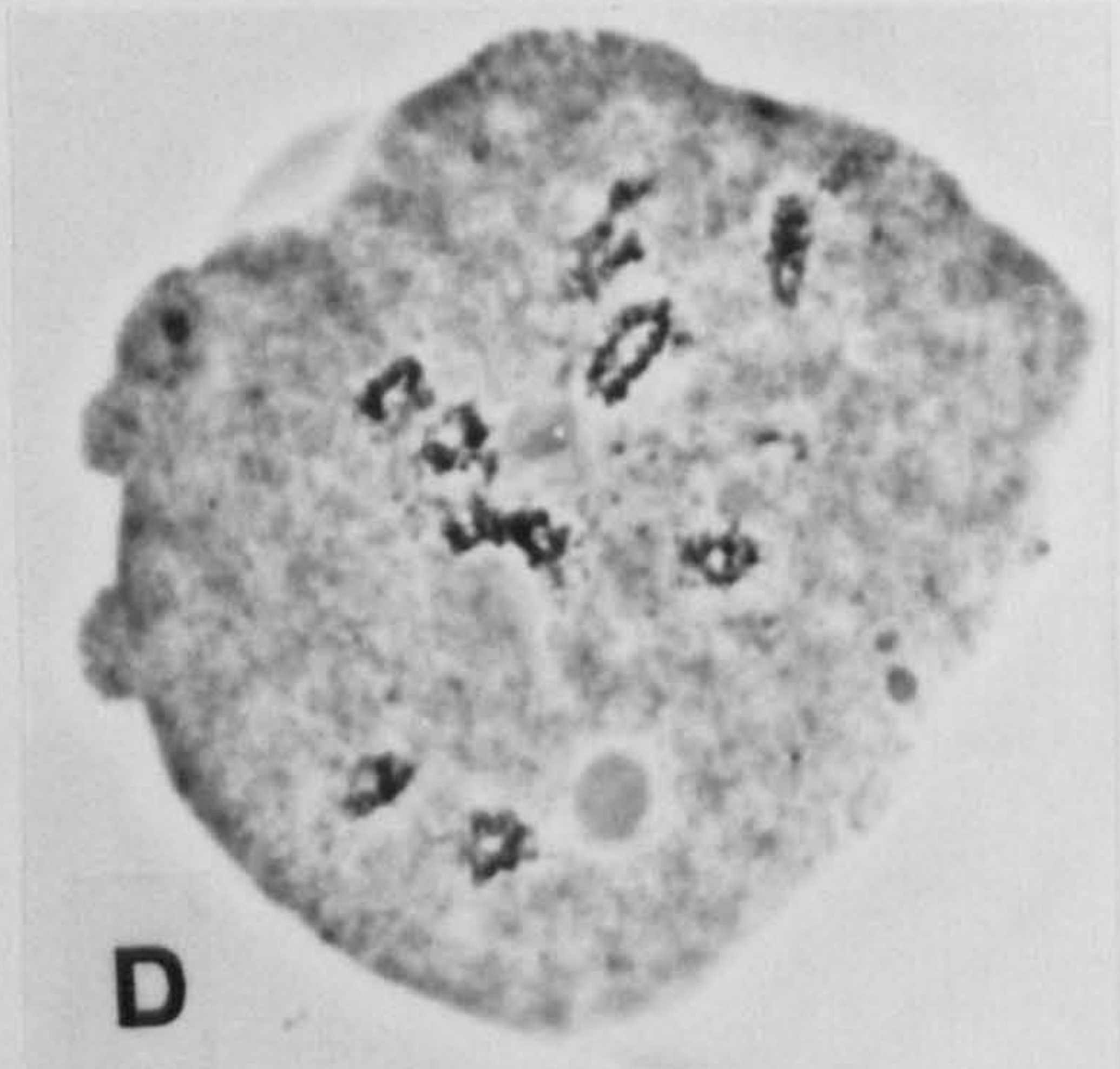
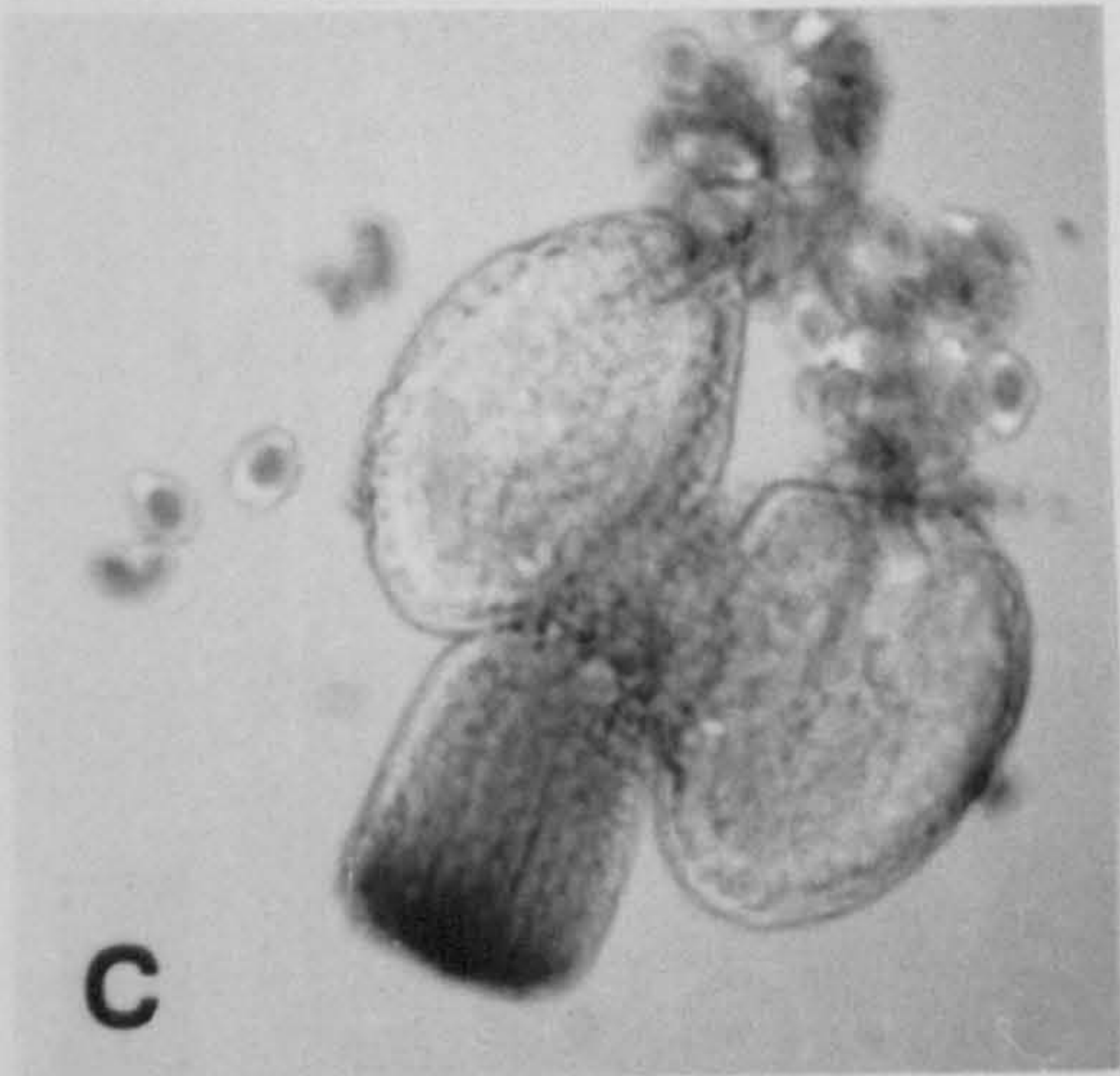
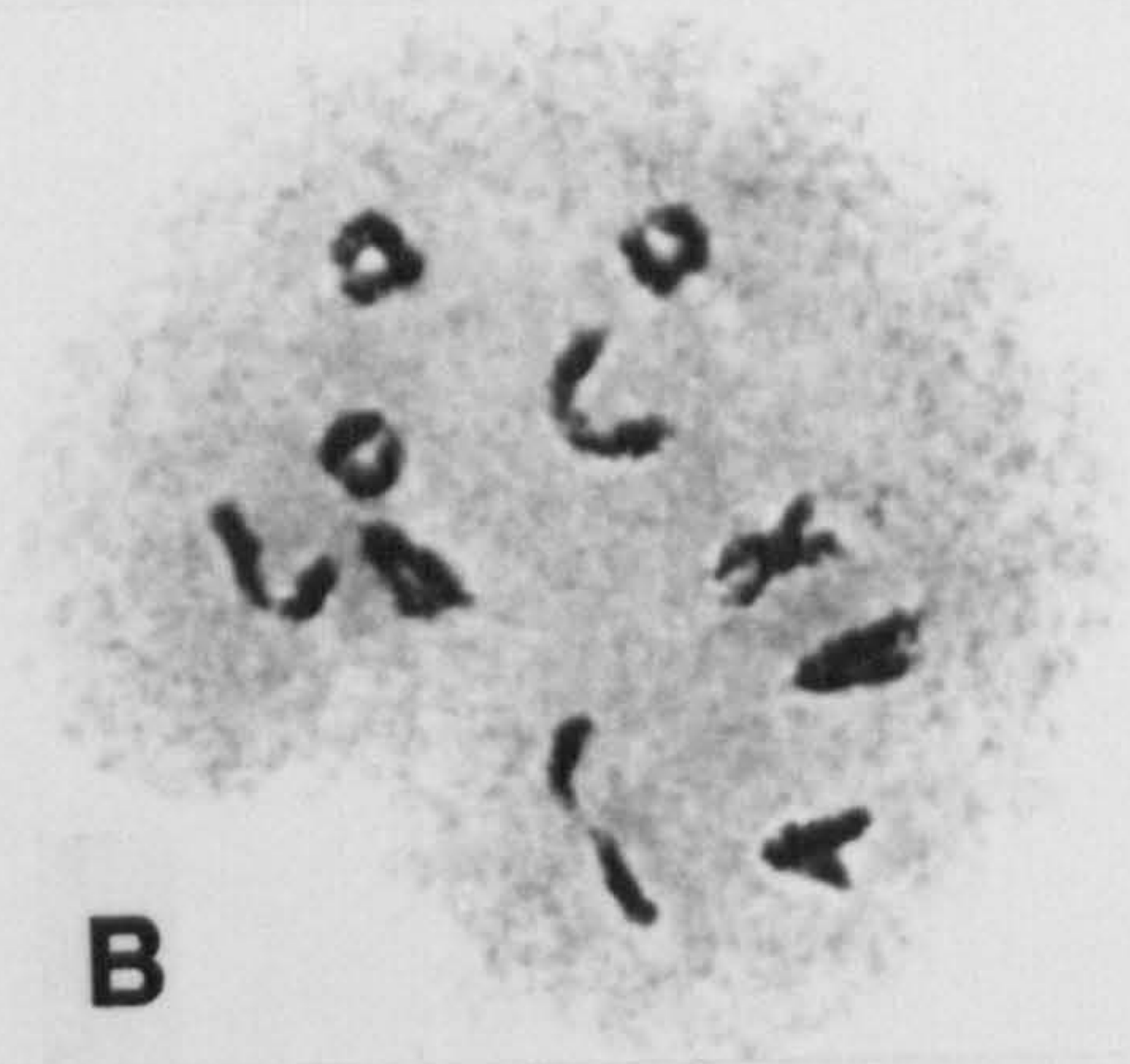
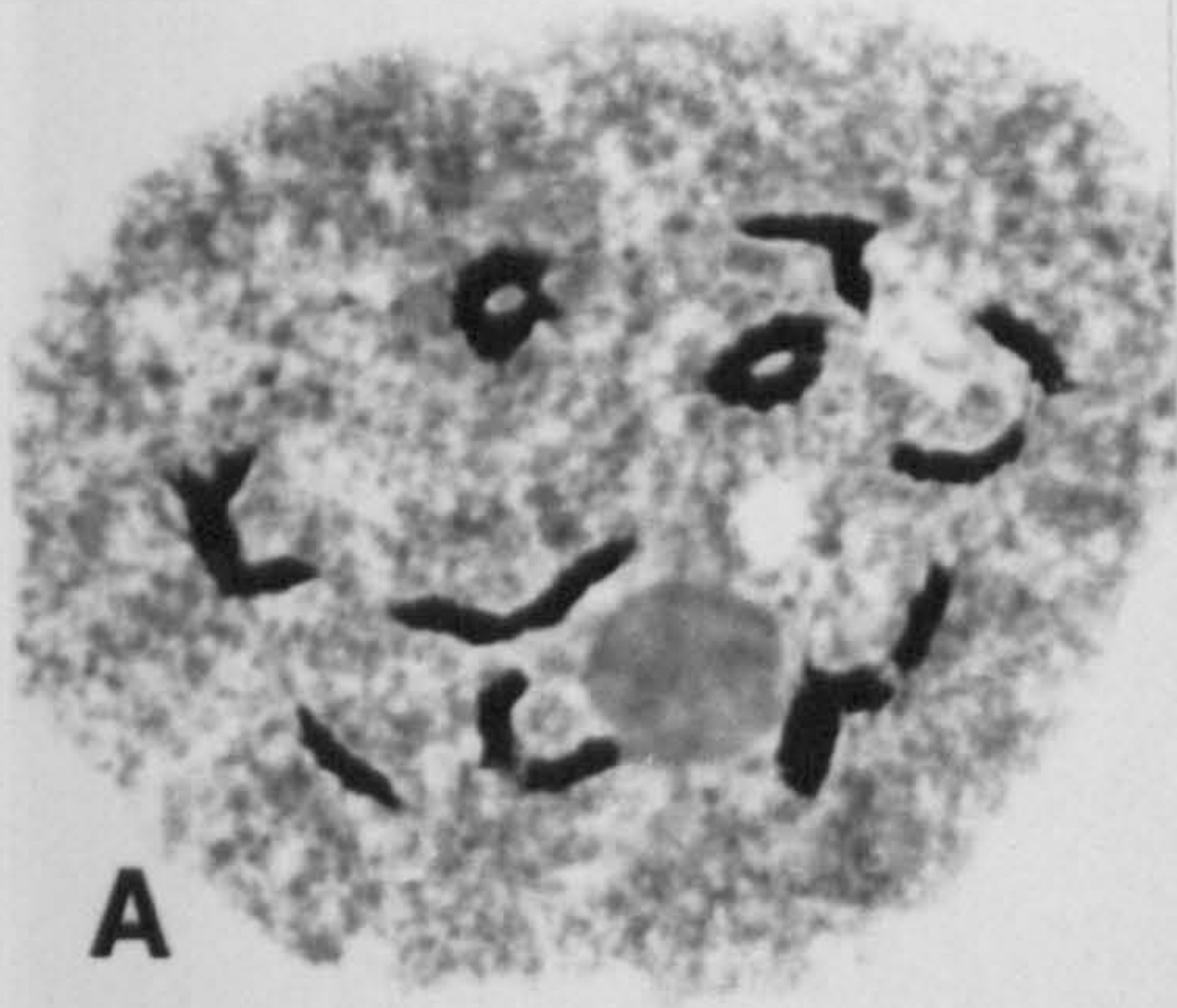


Plate 3.9

Diploid meiosis

A,B *F. baldschuanica* P174
10 II

C *F. scandens* var. *dumetorum*
anther and PMCs X 150

D *F. scandens* var. *dumetorum*
P177 10II

E,F *F. cilinodis* P148 10II

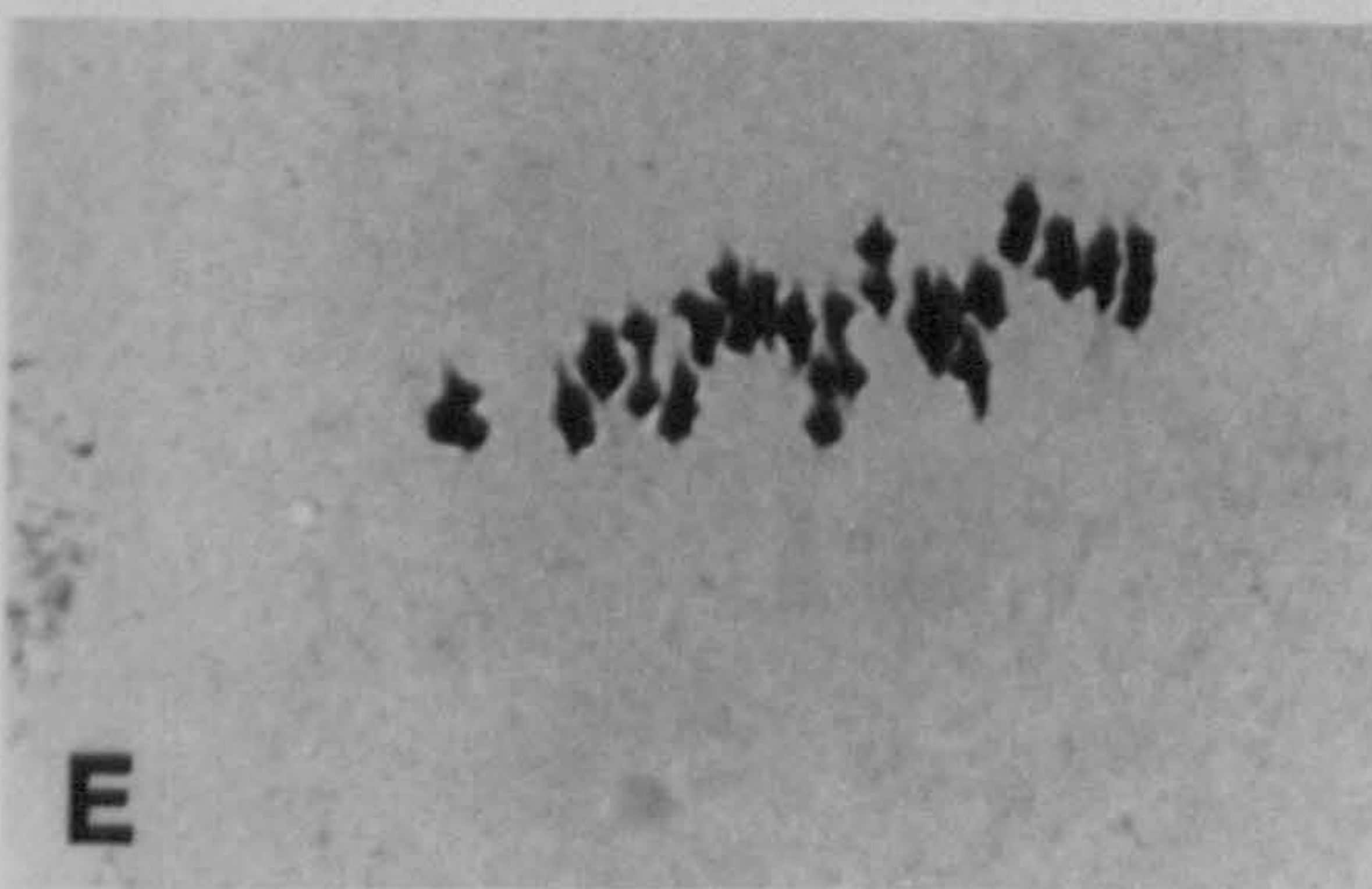
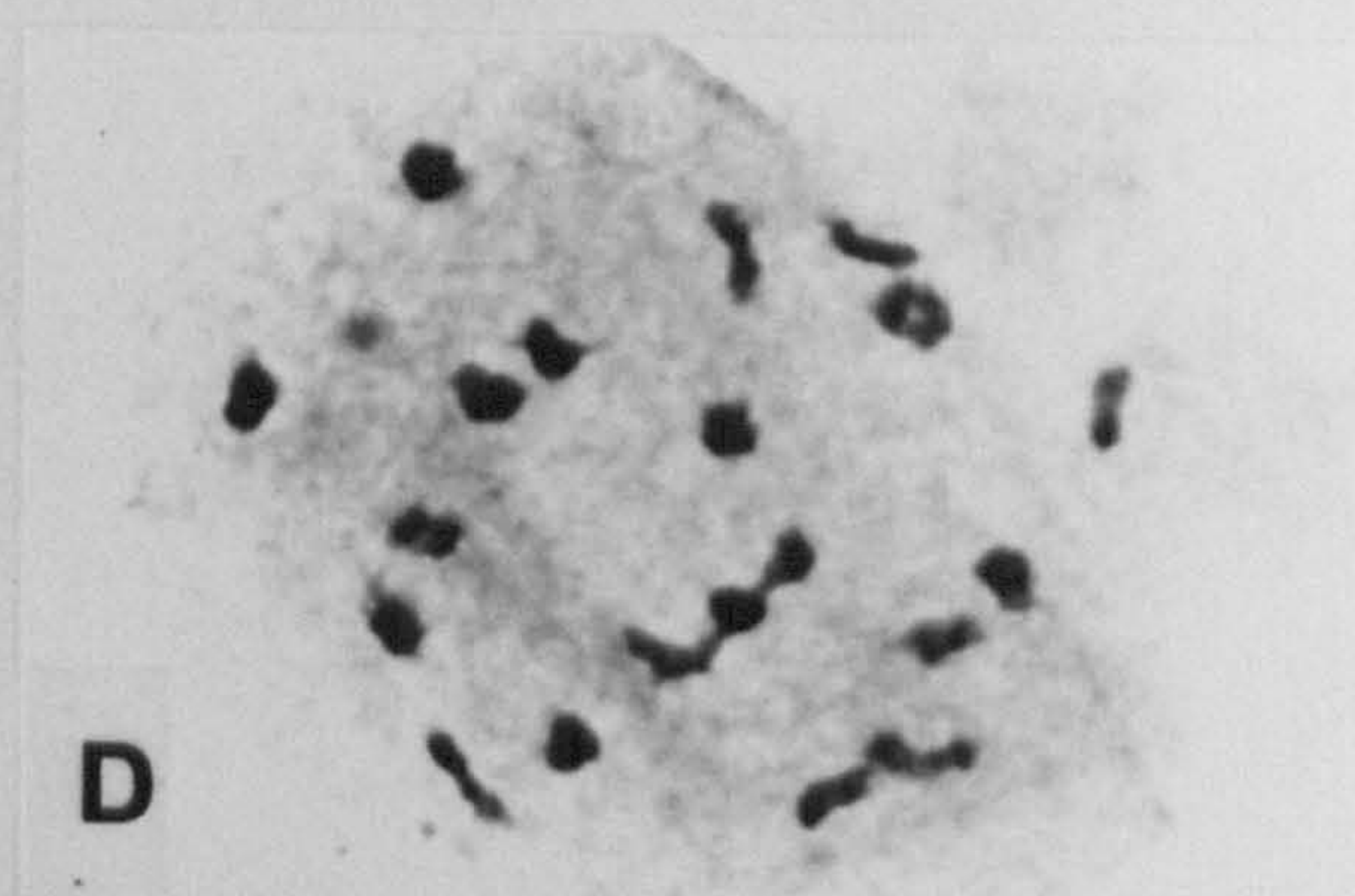
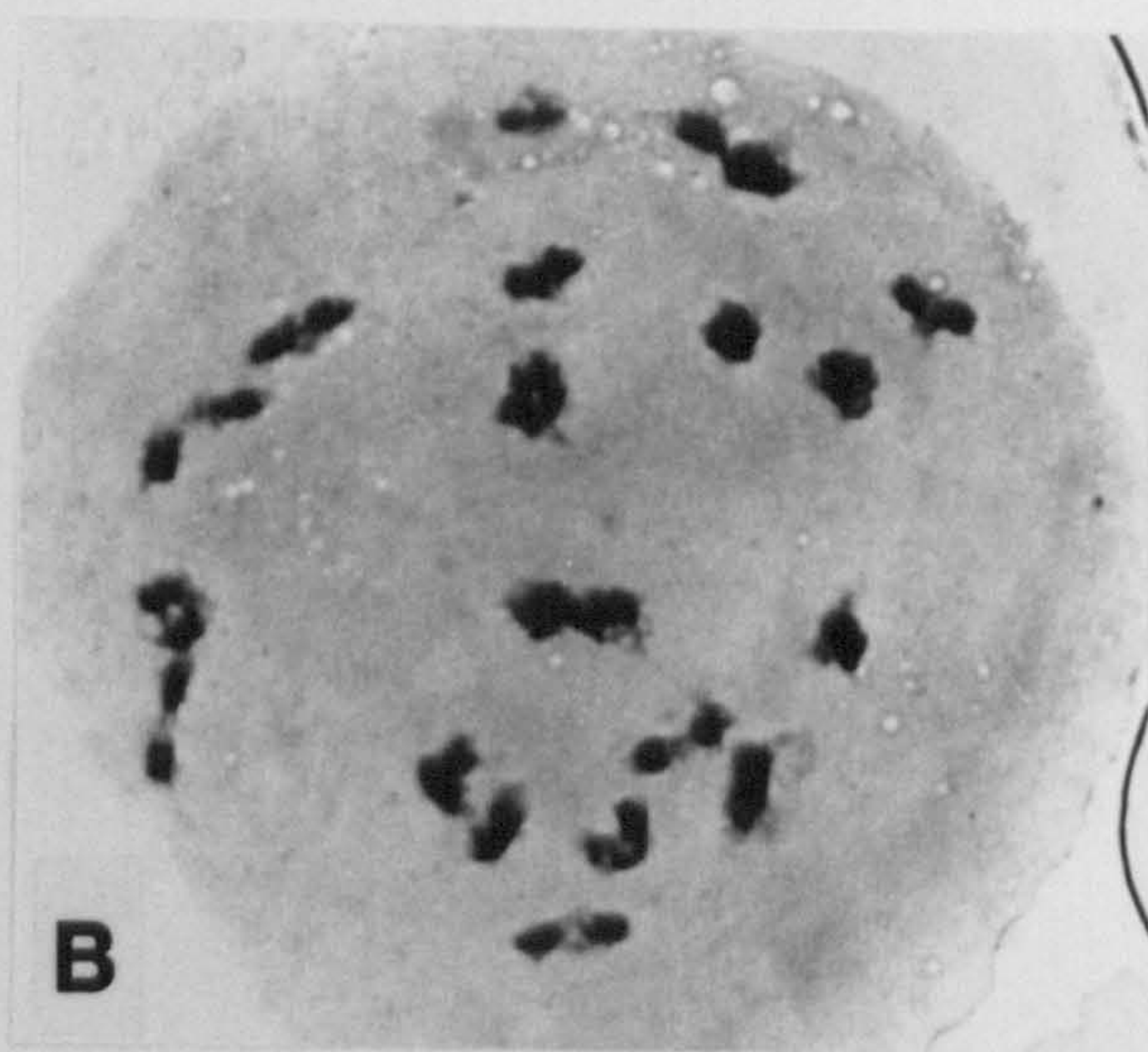
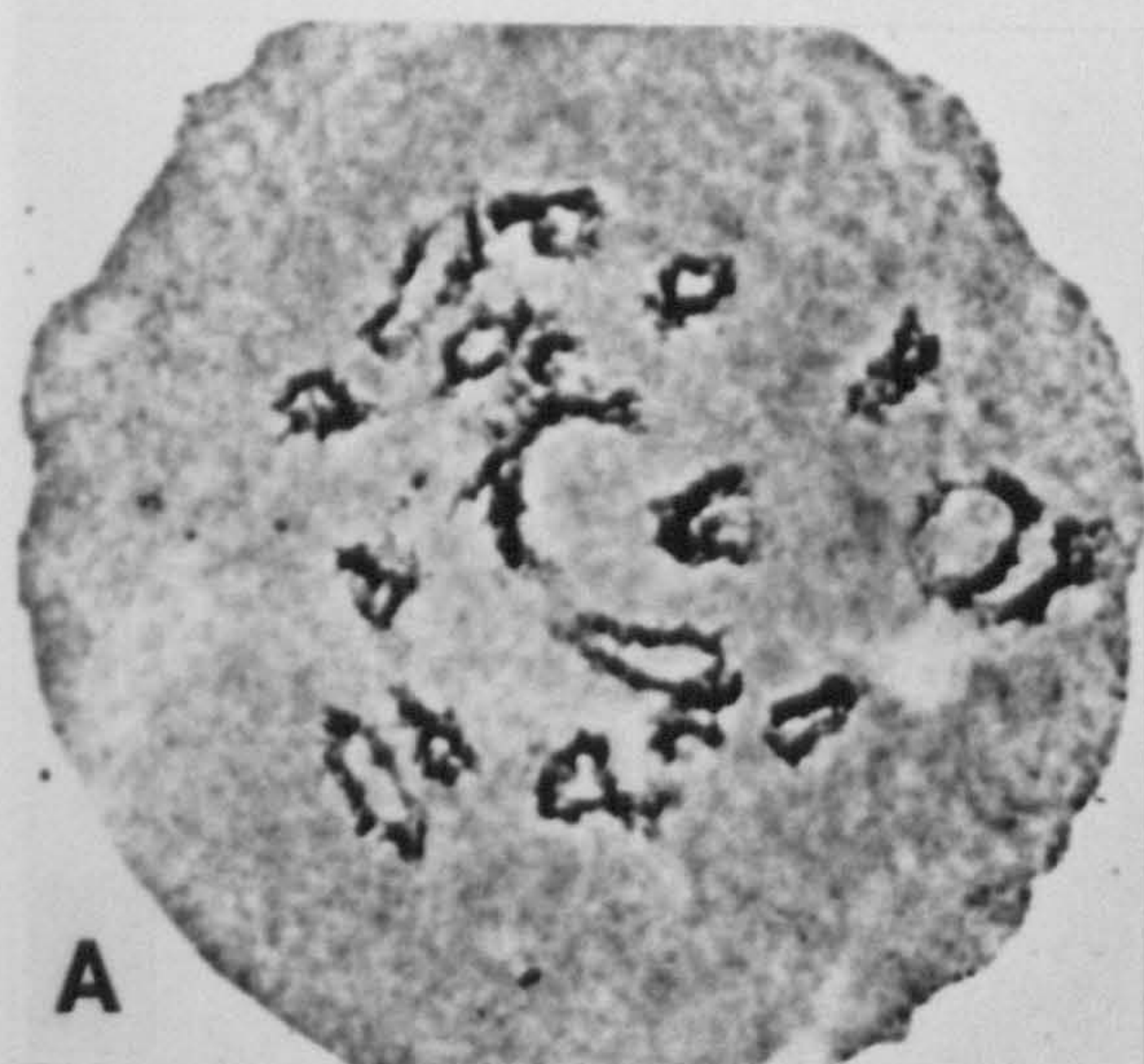


Plate 3.10 *F.convolvulus* P178 Meiosis

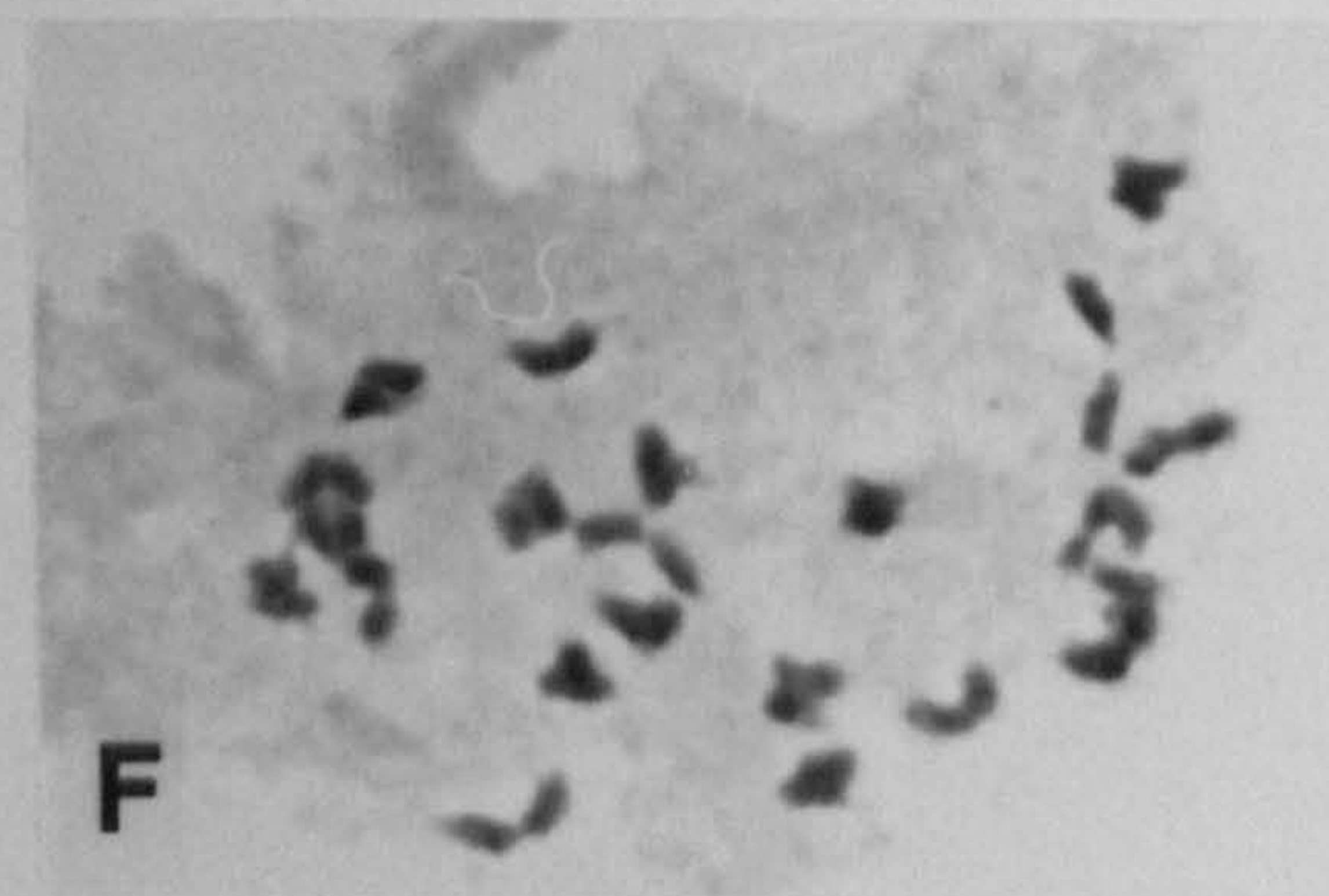
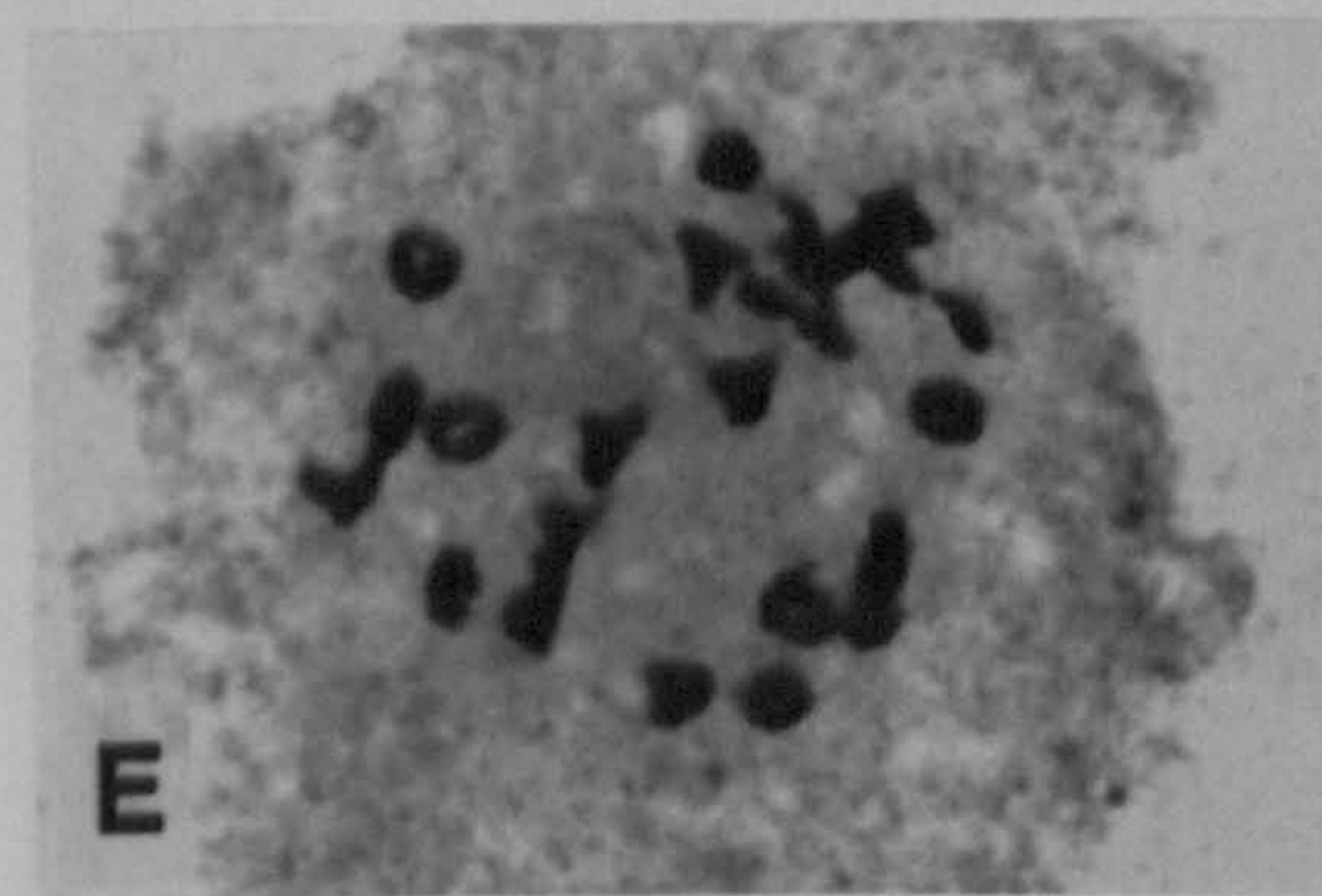
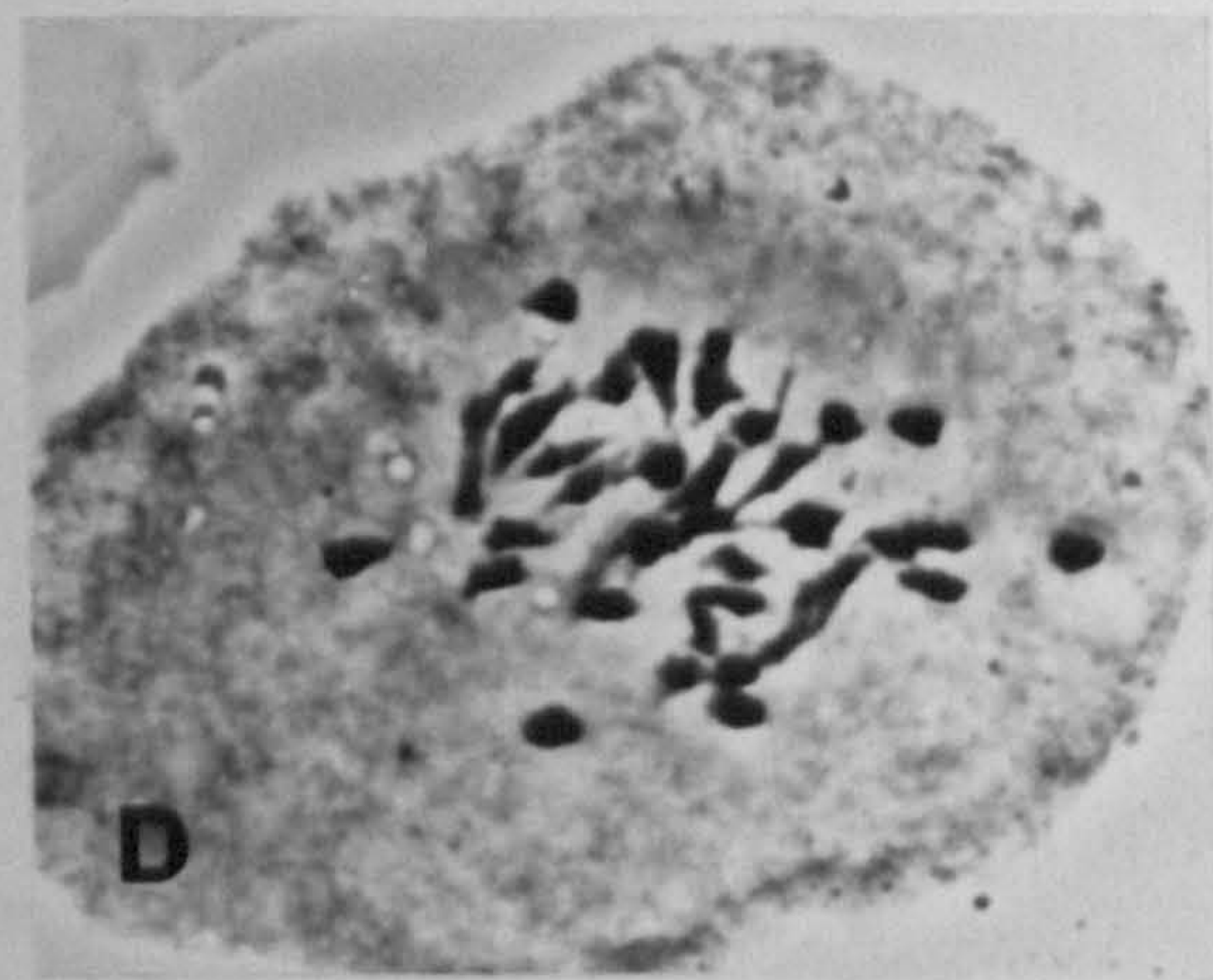
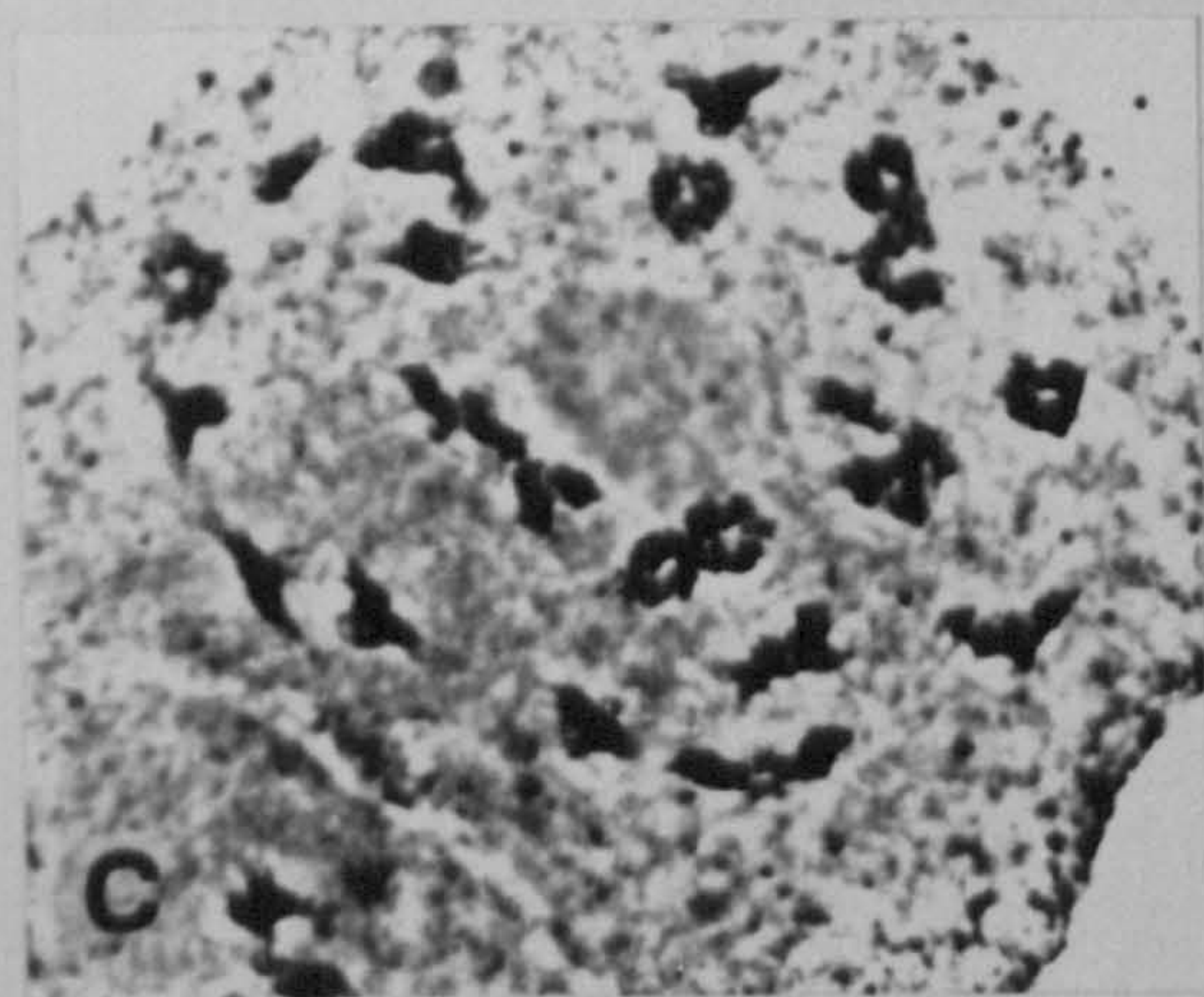
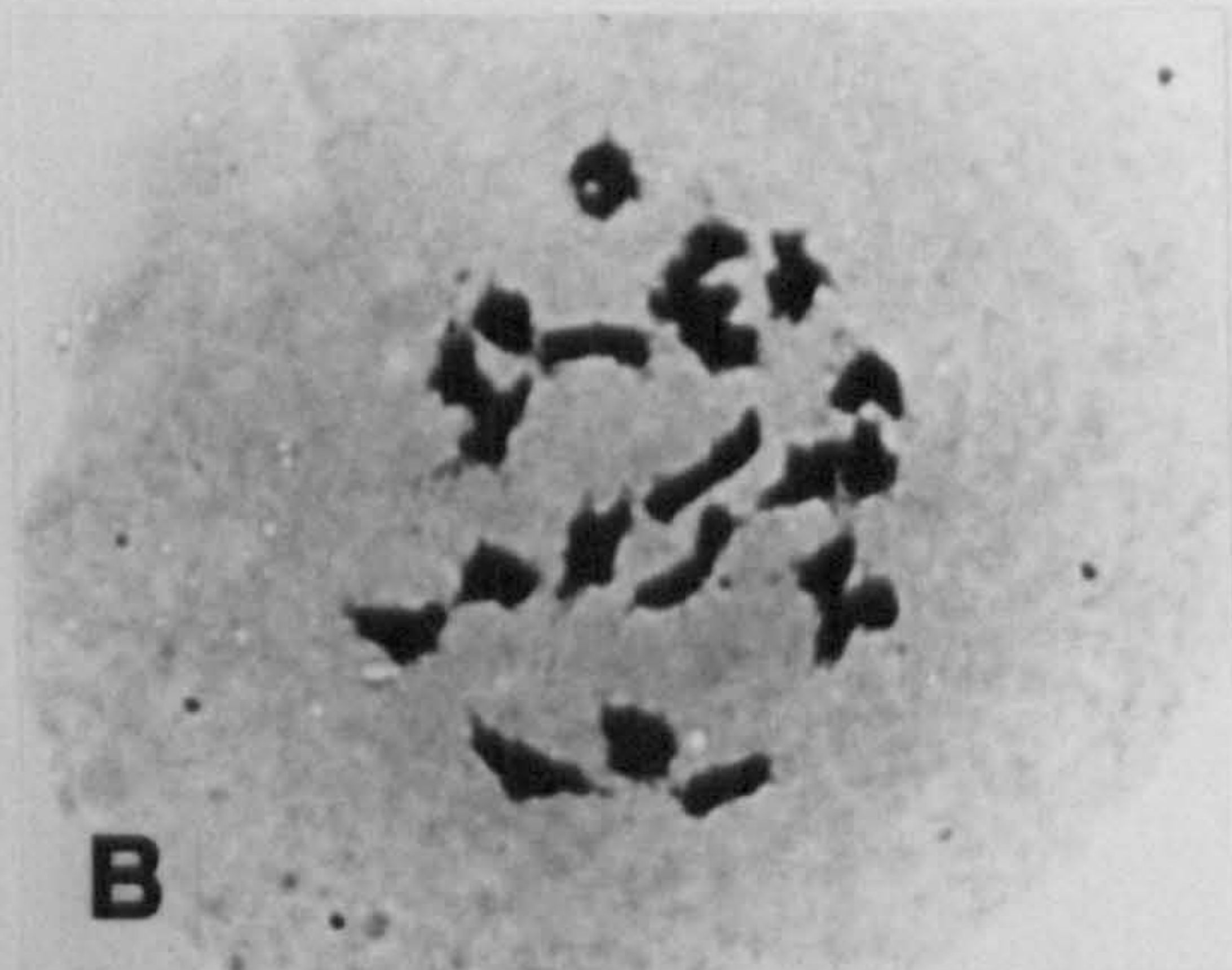
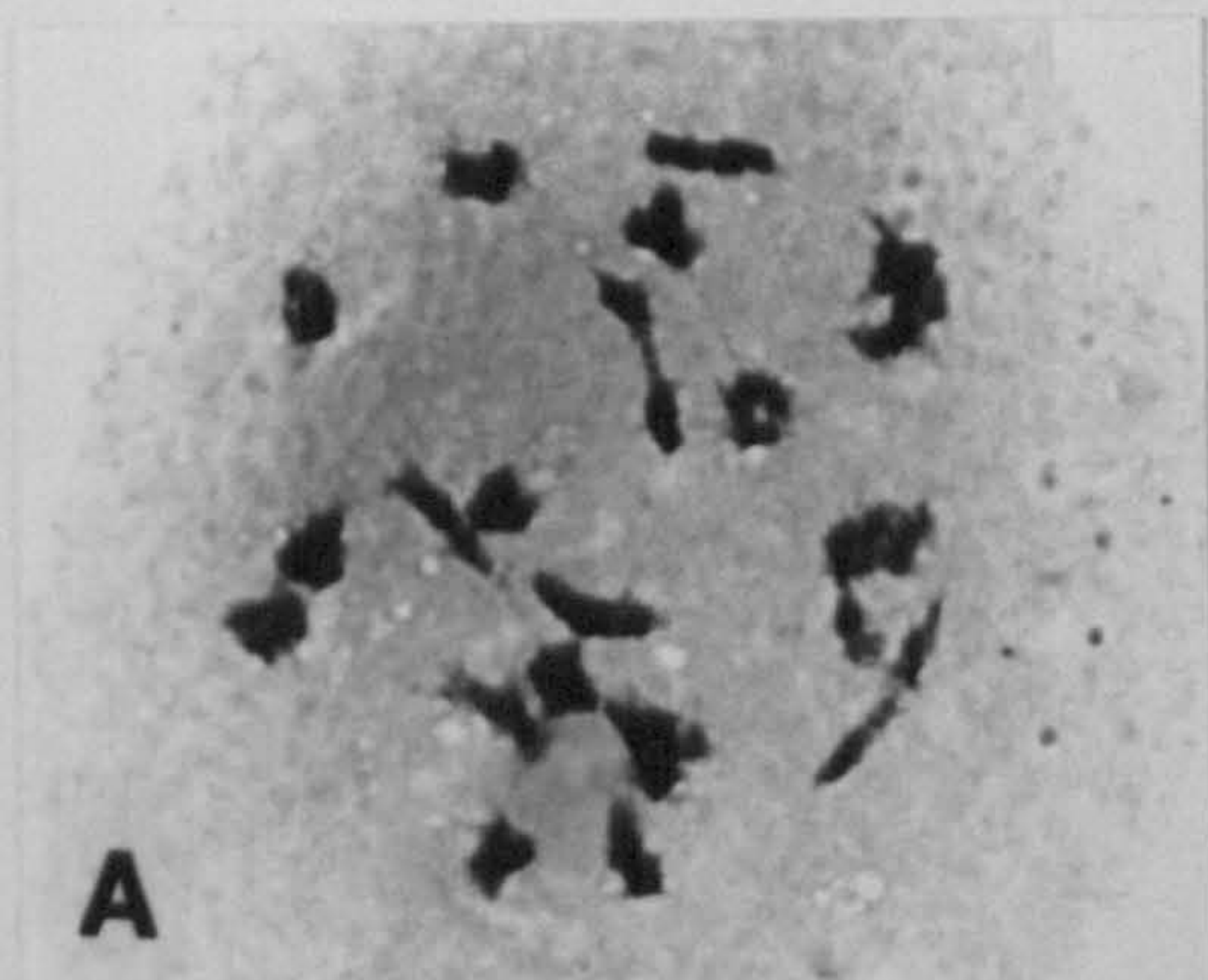


Plate 3. 11 Tetraploid Meiosis

A-D *R. japonica* var. *compactum* P2a

A,B showing good bivalent formation

C showing multivalent formation

D irregular anaphase I from the same preparation as C

E,F *R. japonica* P114b showing regular bivalent formation

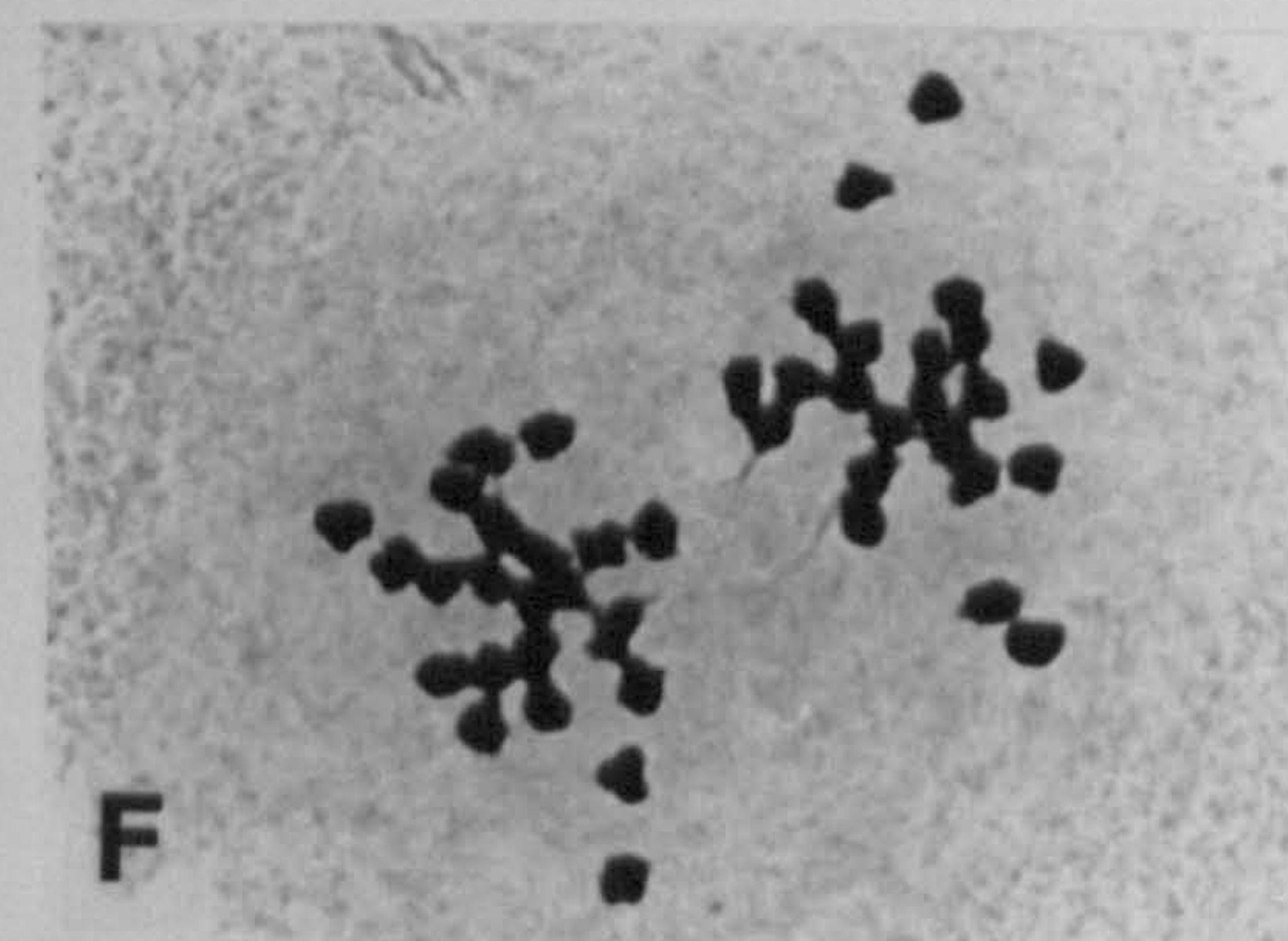
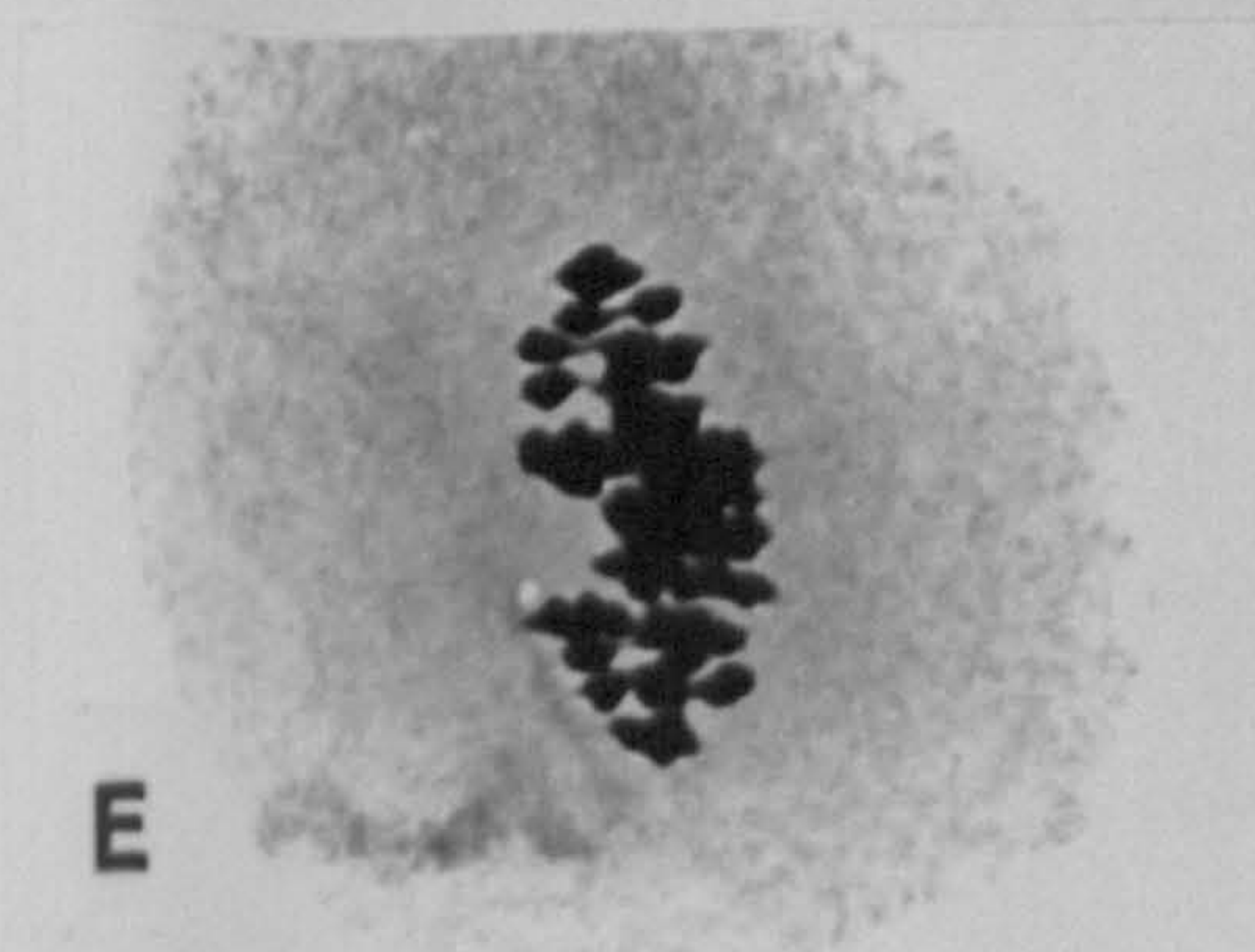
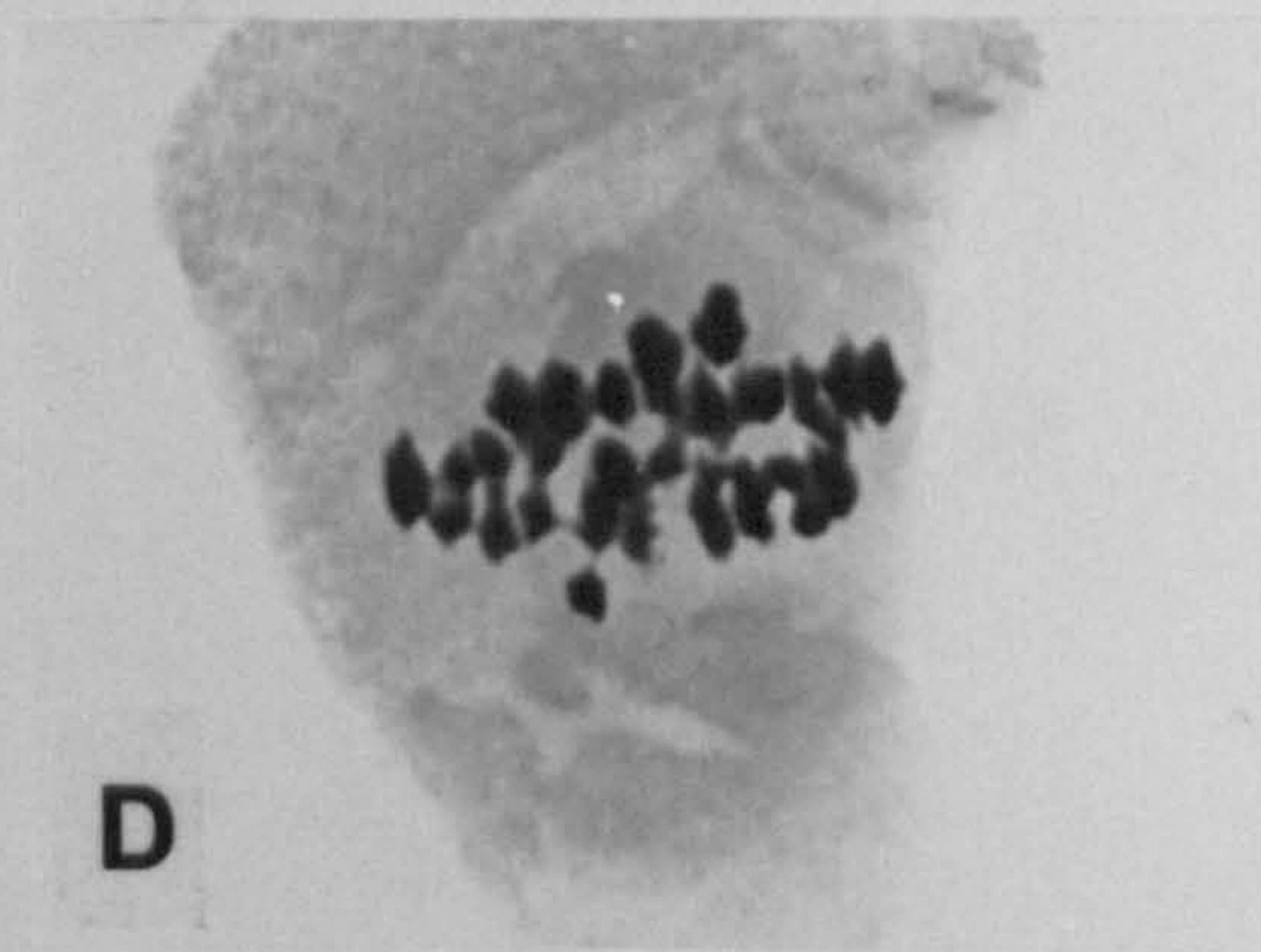
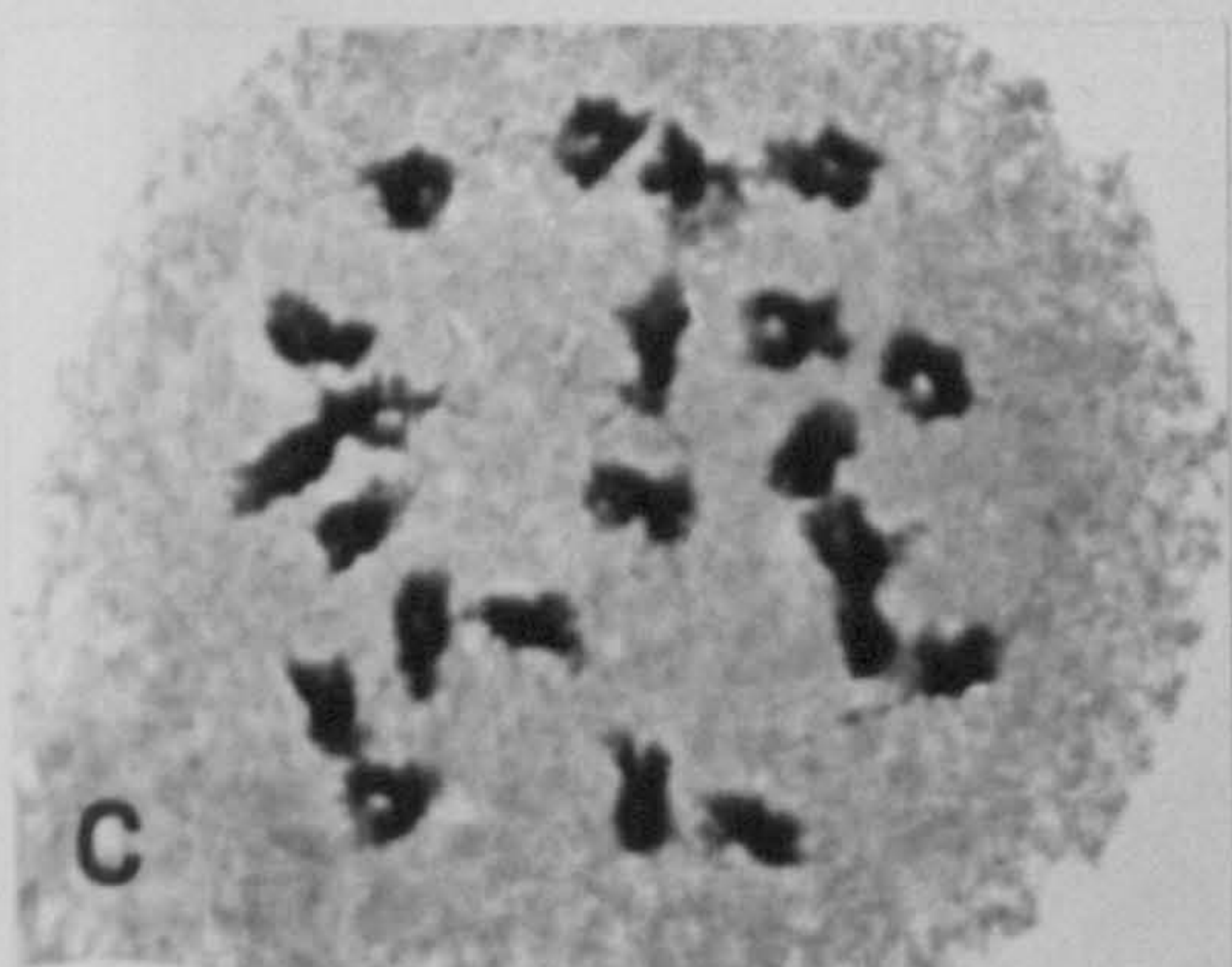
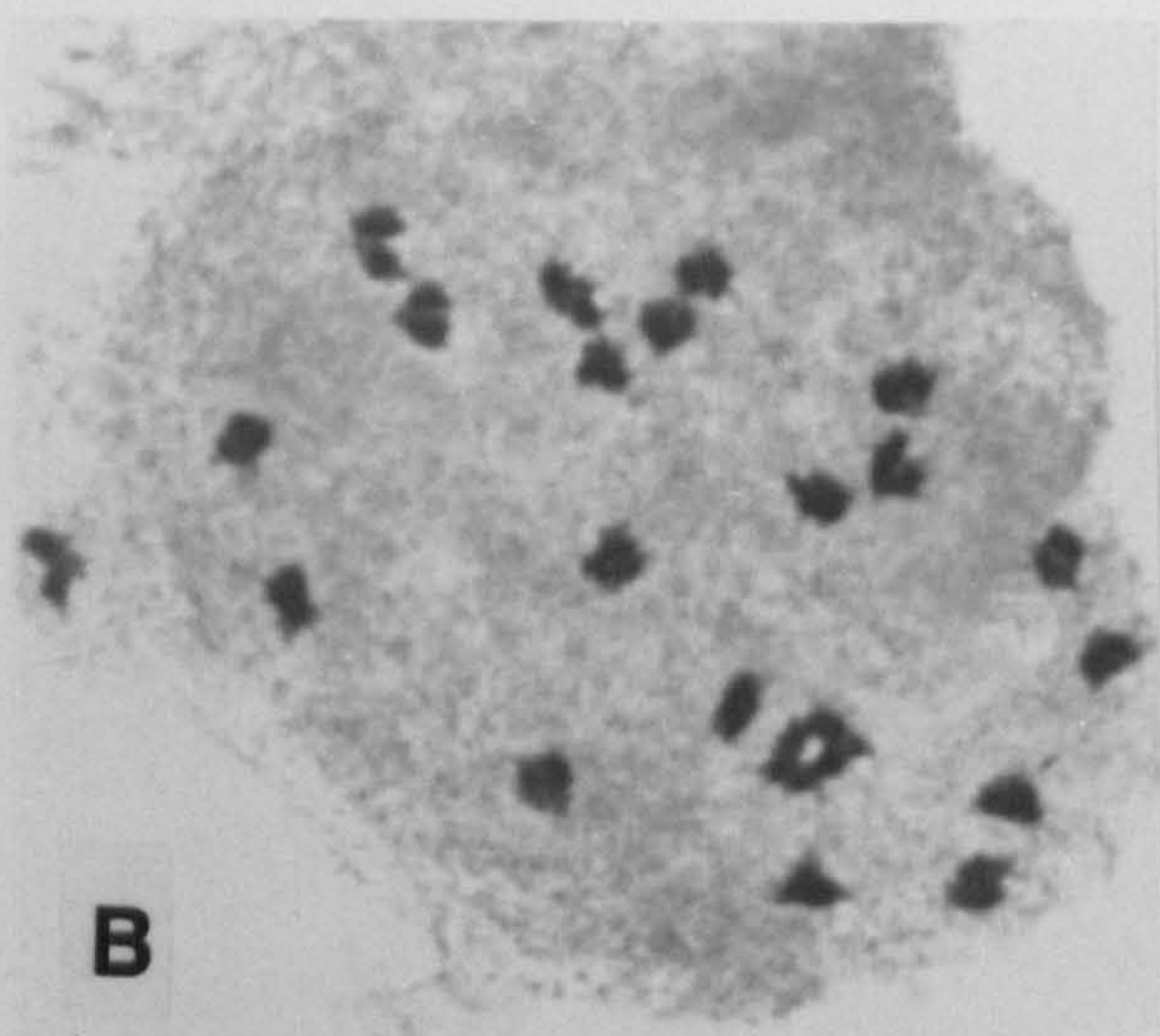
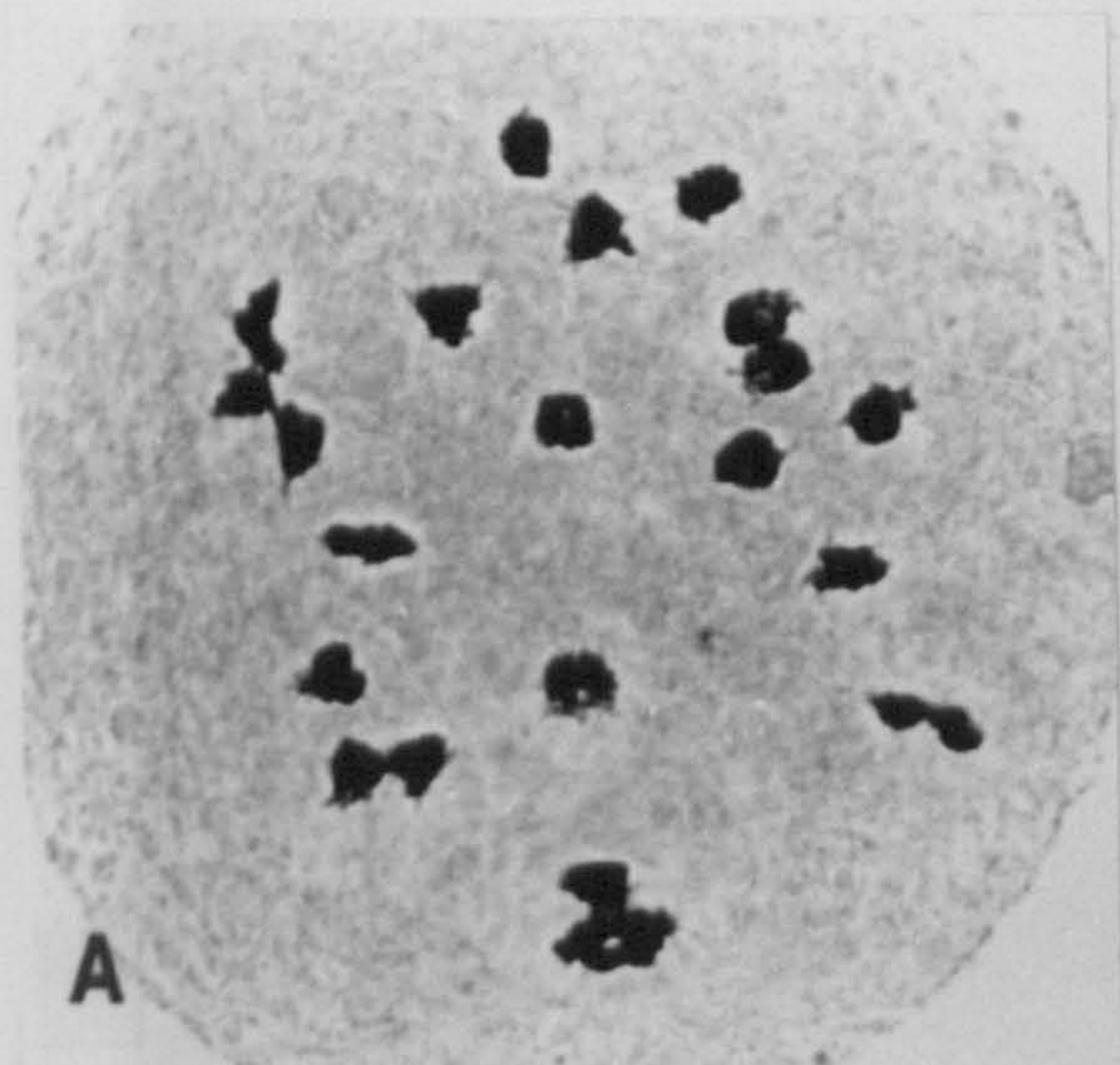


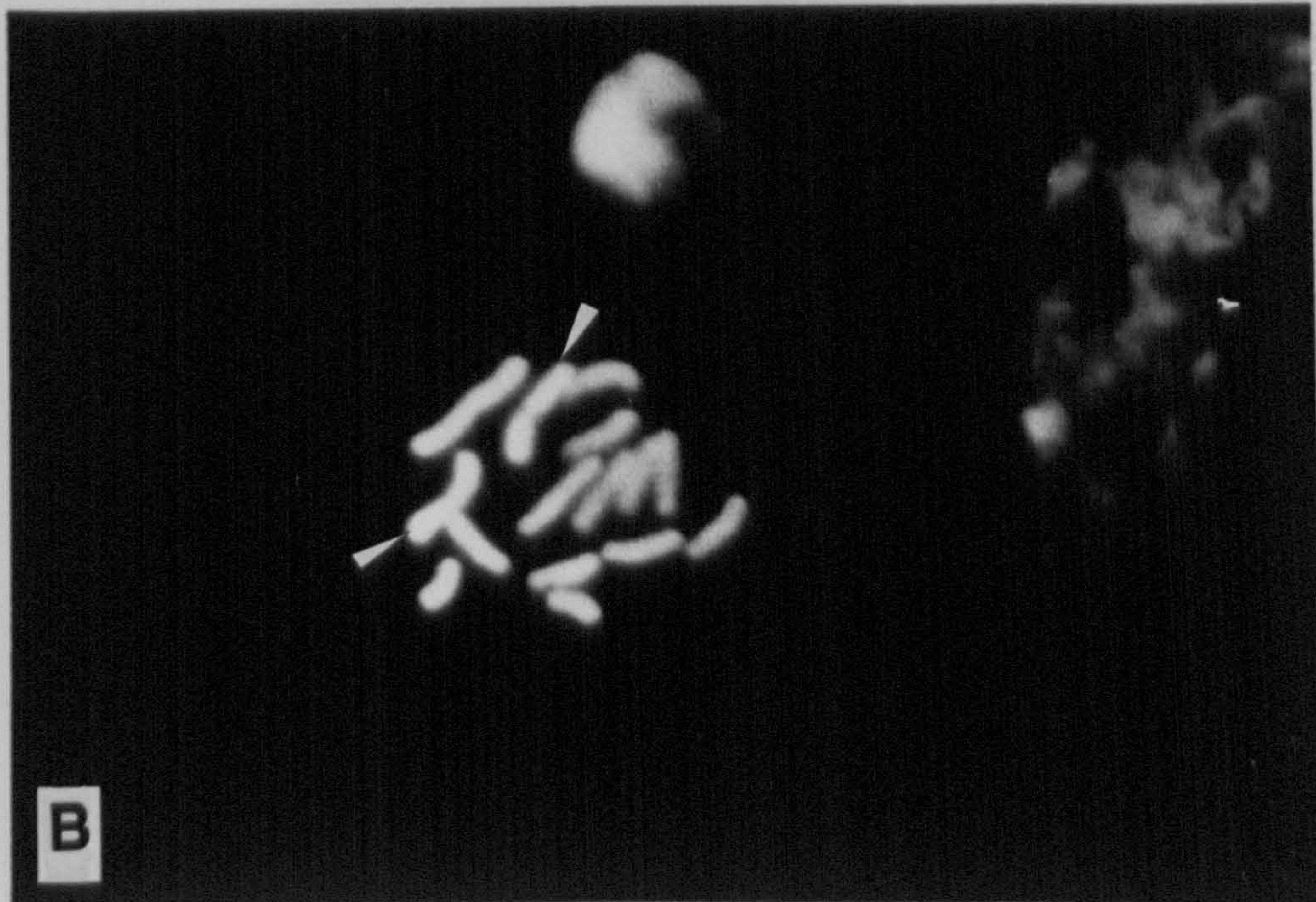
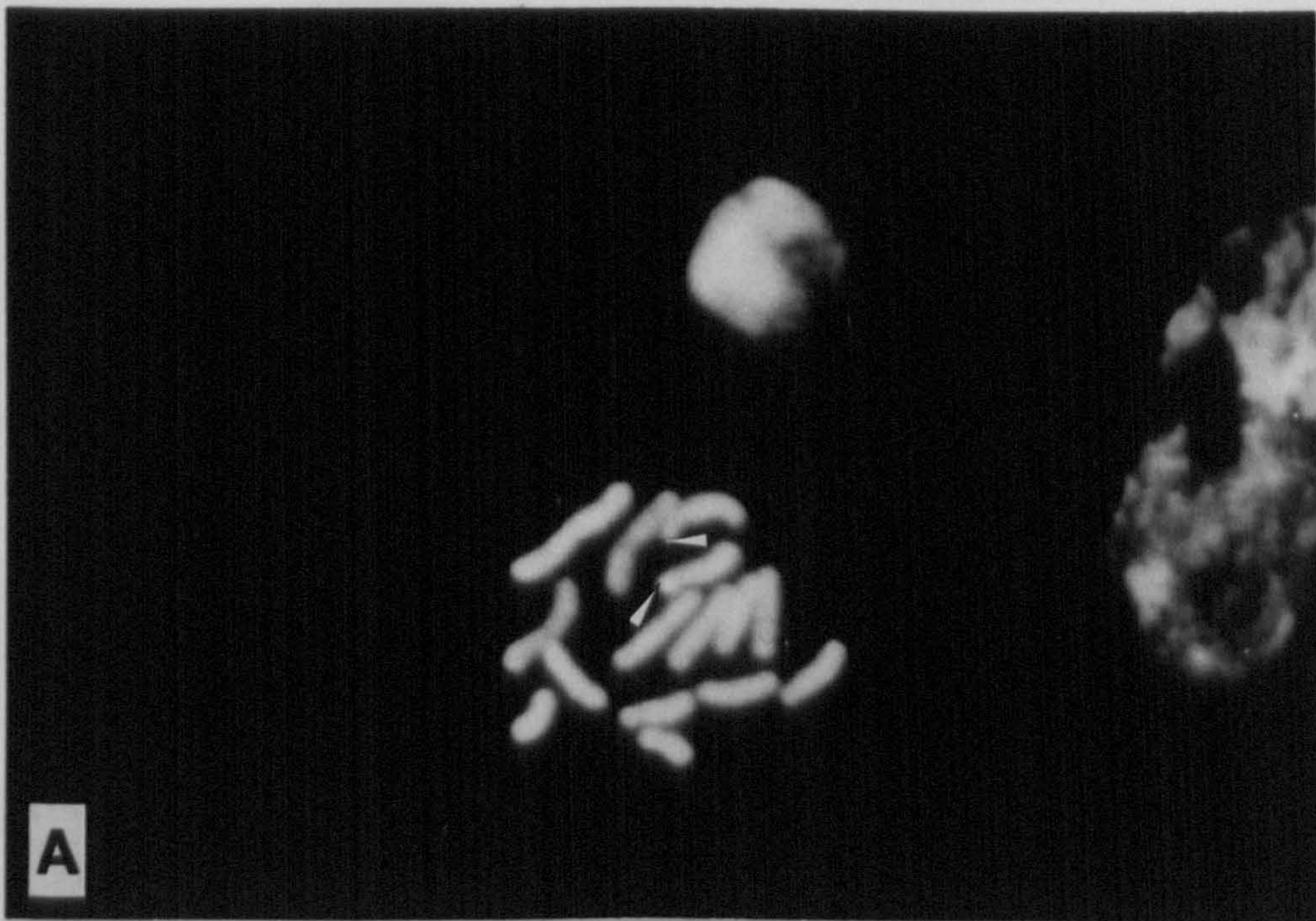
Plate 3.12 Tetraploid meiosis
R. sachalinensis P68 $2n = 44$

A, B diakinesis with 20II and one ring IV

C diakinesis showing regular bivalent formation

D, E metaphase I

F telophase I to show regular segregation



**Plate 3. 13 Chromomycin/DAPI staining of
Rumex acetosa (male)**

- A** Cell viewed by chromomycin fluorescence, note the Y chromosomes showing diminished fluorescence and the positive fluorescence of the satellites. (arrowed).
- B** Same cell viewed by DAPI fluorescence note enhanced fluorescence of the Y chromosomes (arrowed), and the unstained satellites.

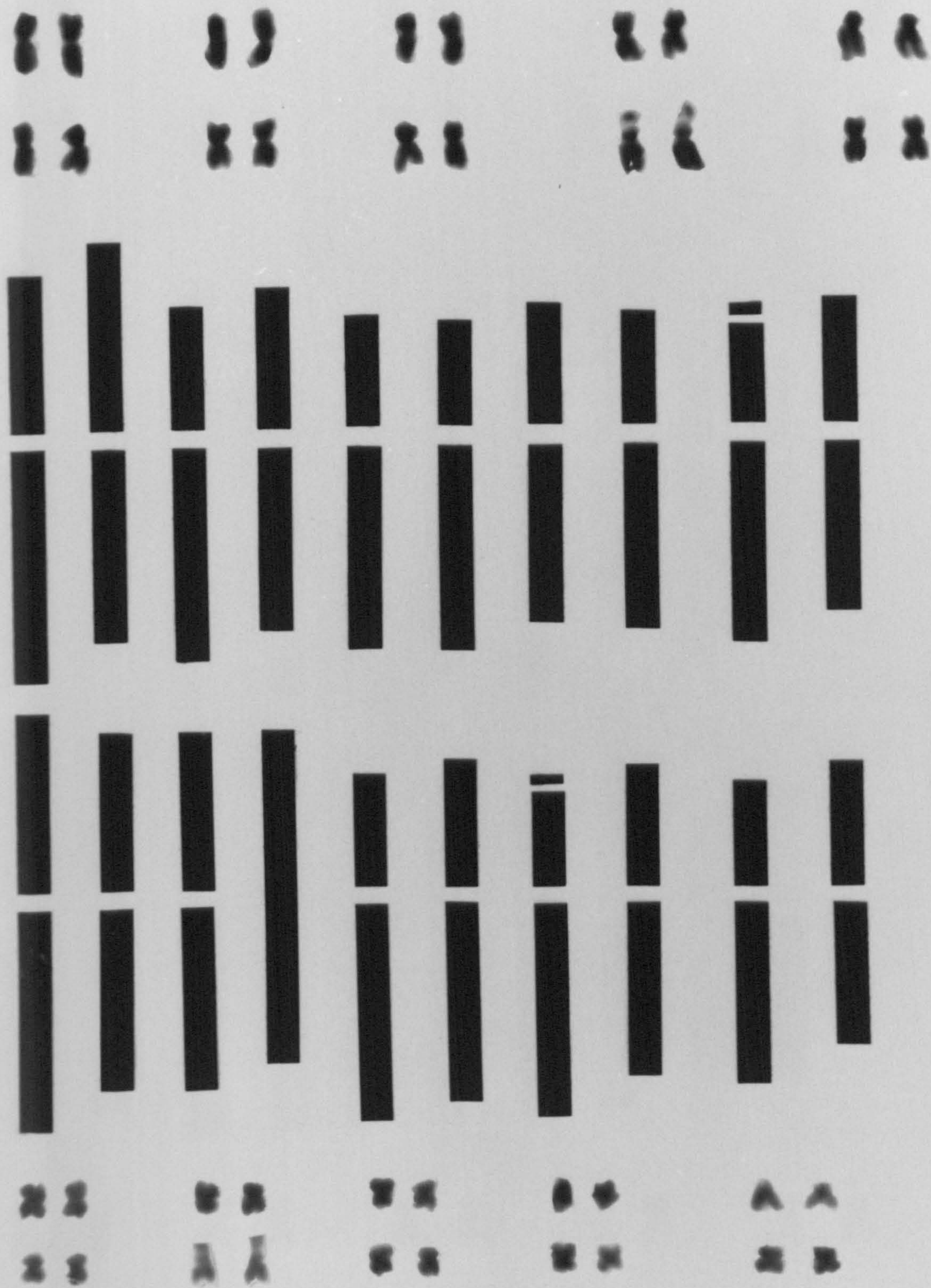


Figure 3.1

Karyotypes and karyograms of two cells of *F. scandens* var. *dumetorum* P177 $2n = 20$.

Chromosomes magnified X3000 in this and the following karyotypes

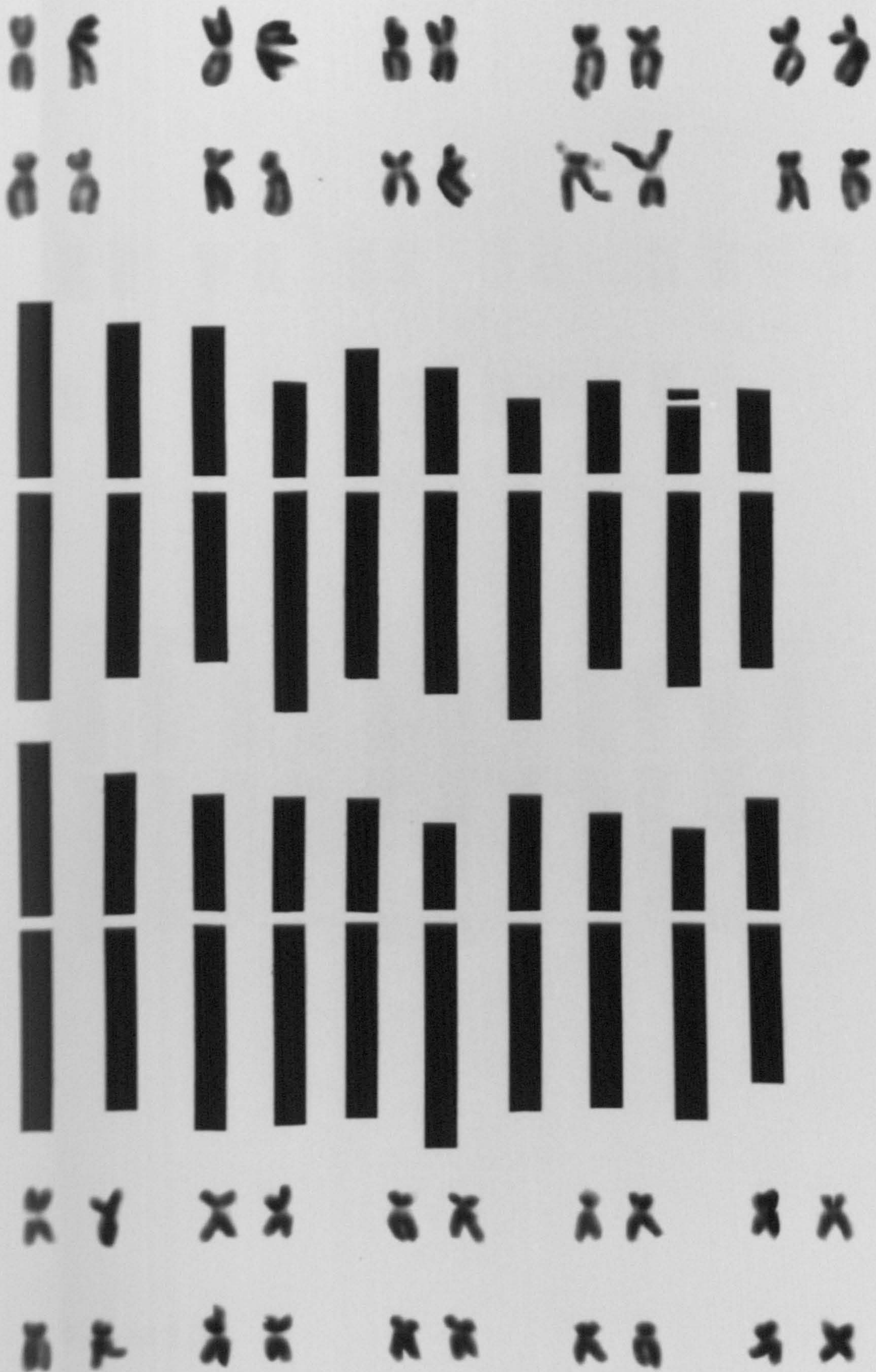


Figure 3.2

Karyotypes and karyograms of two cells of *F. baldschuanica* P163
 $2n = 20$

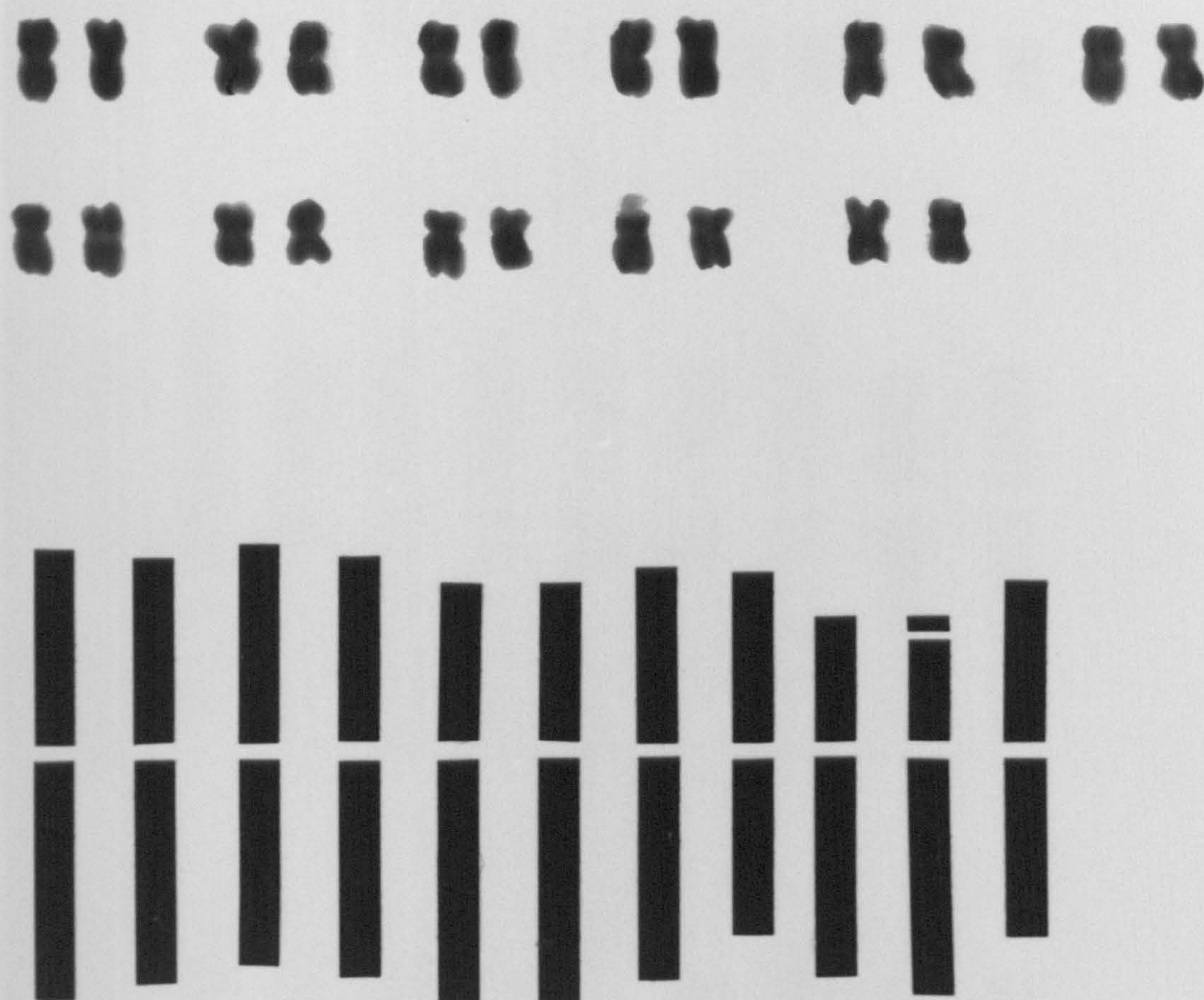


Figure 3.3

Karyotype and karyogram of *F. cilinodis* P148 $2n = 22$

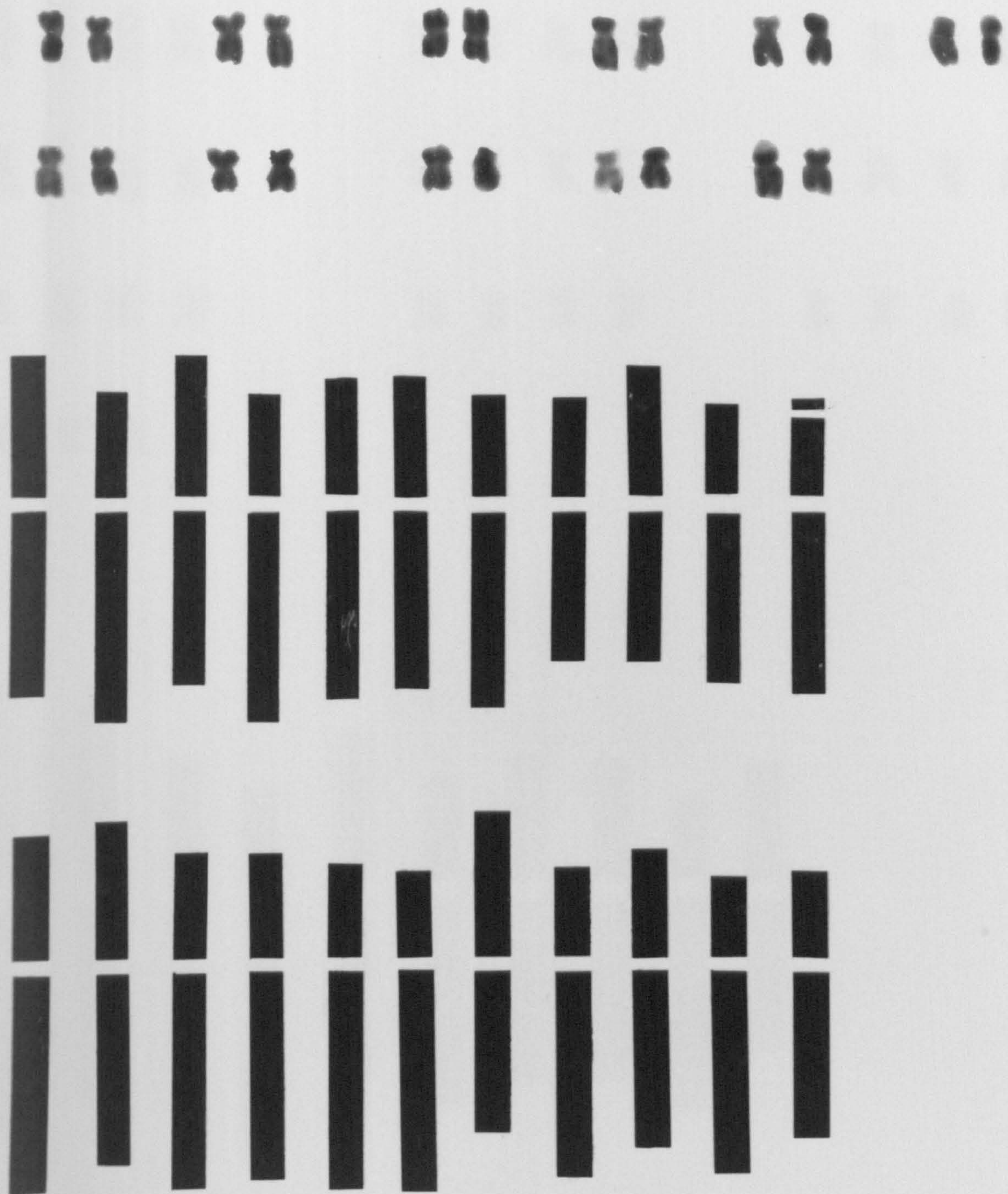


Figure 3.4

Karyotype and karyogram of one cell and karyogram of a second cell of *F. multiflora* P162 $2n = 22$

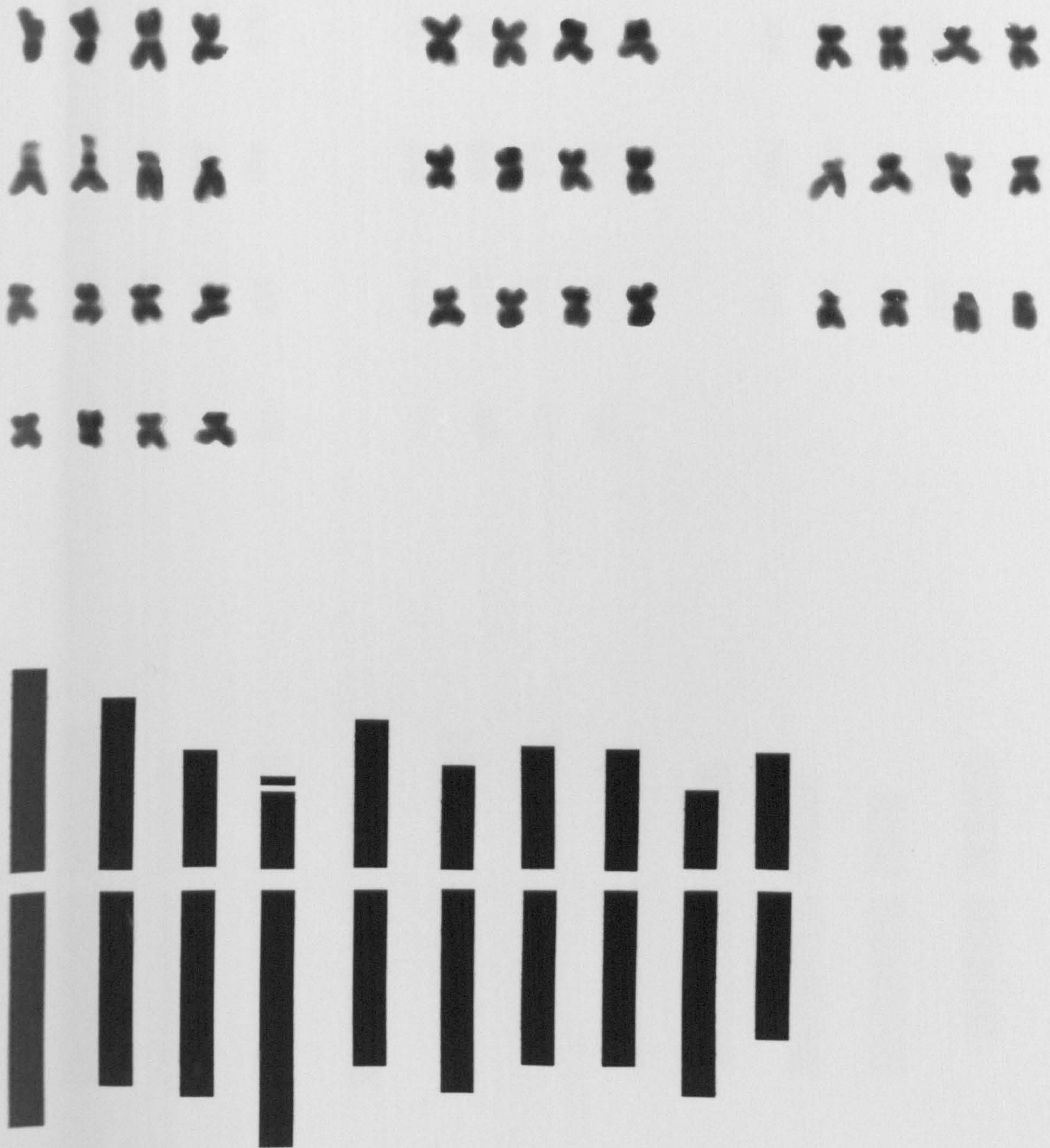


Figure 3.5

Karyotype and karyogram of *F. convolvulus* P150 $2n = 40$

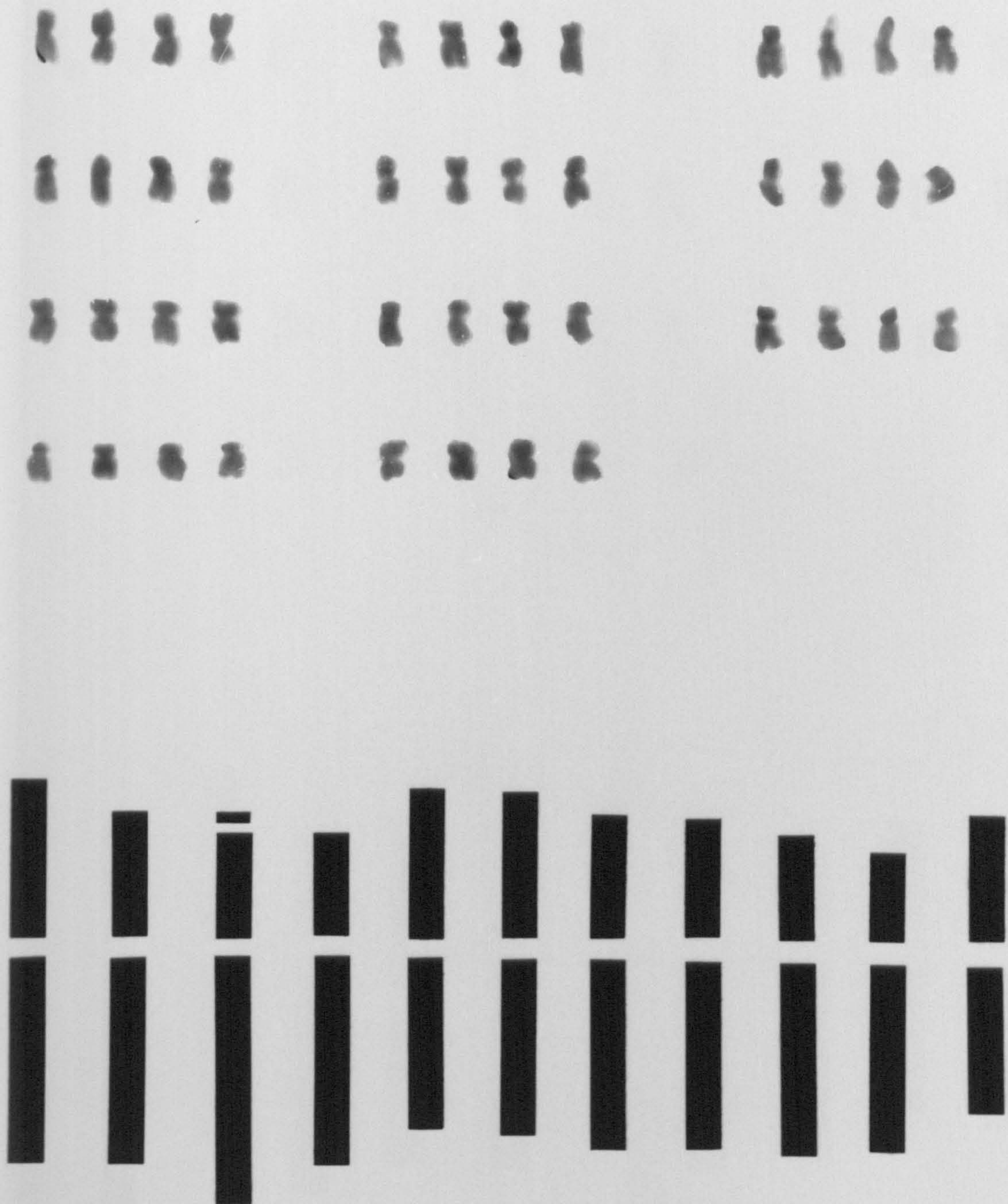


Figure 3.6

Karyotype and karyogram of *R. sachalinensis* P155 $2n = 44$

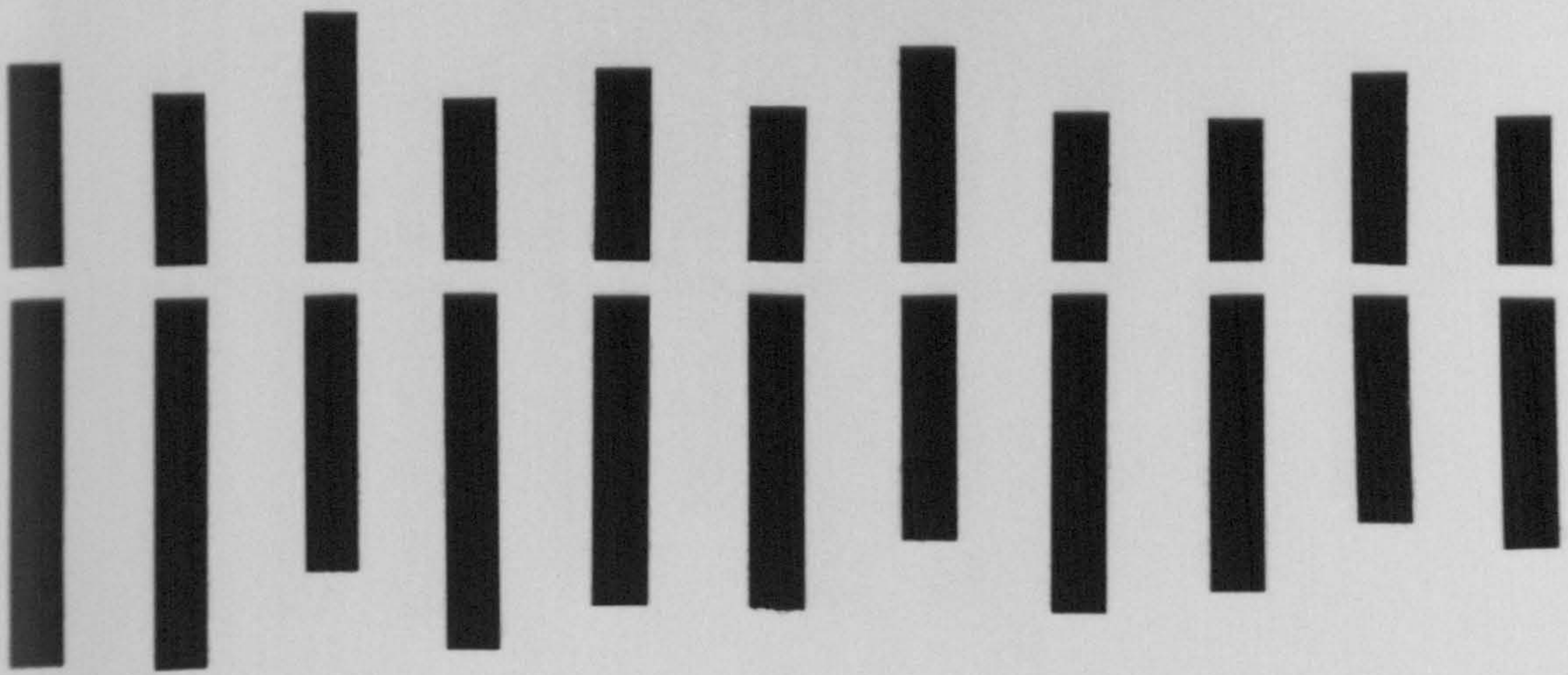


Figure 3.7

Karyotype and karyogram of *R. japonica* var. *compacta* P99a $2n = 44$

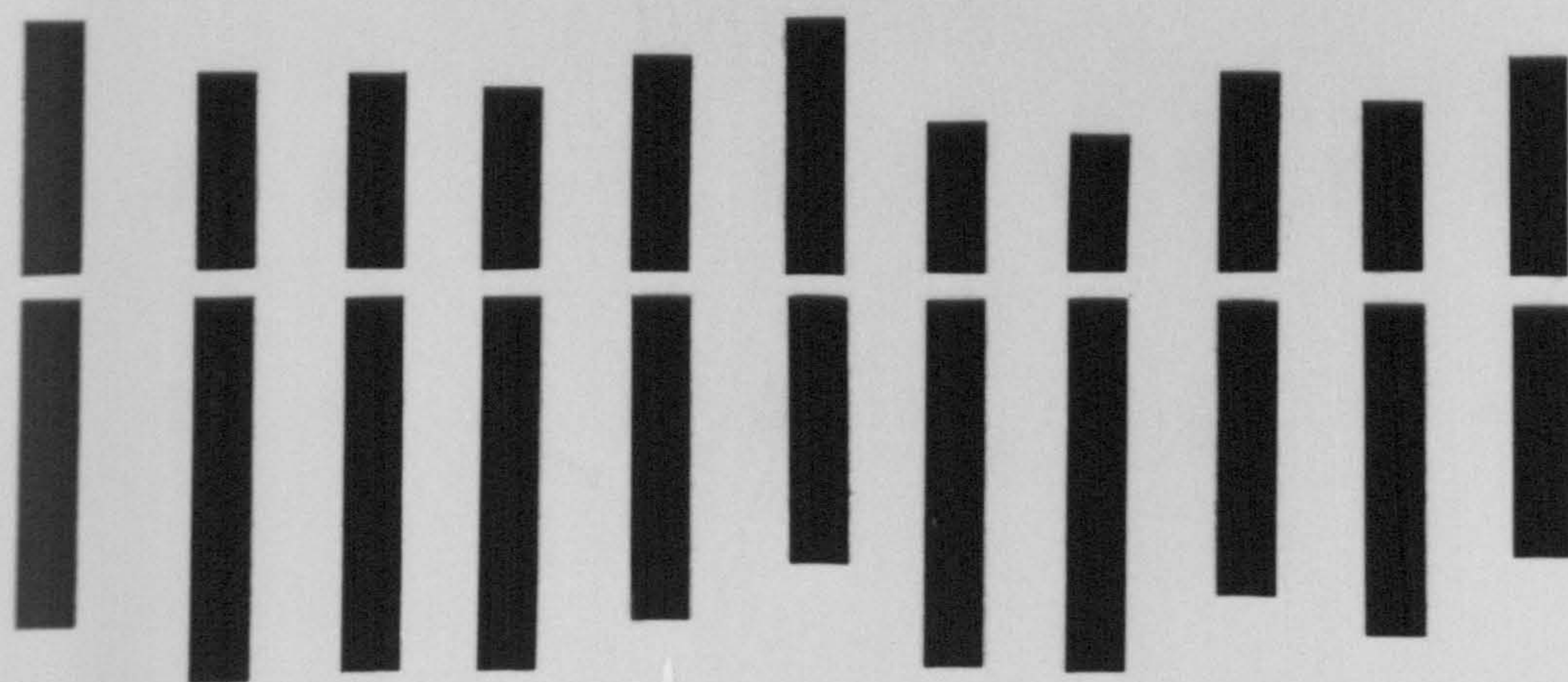
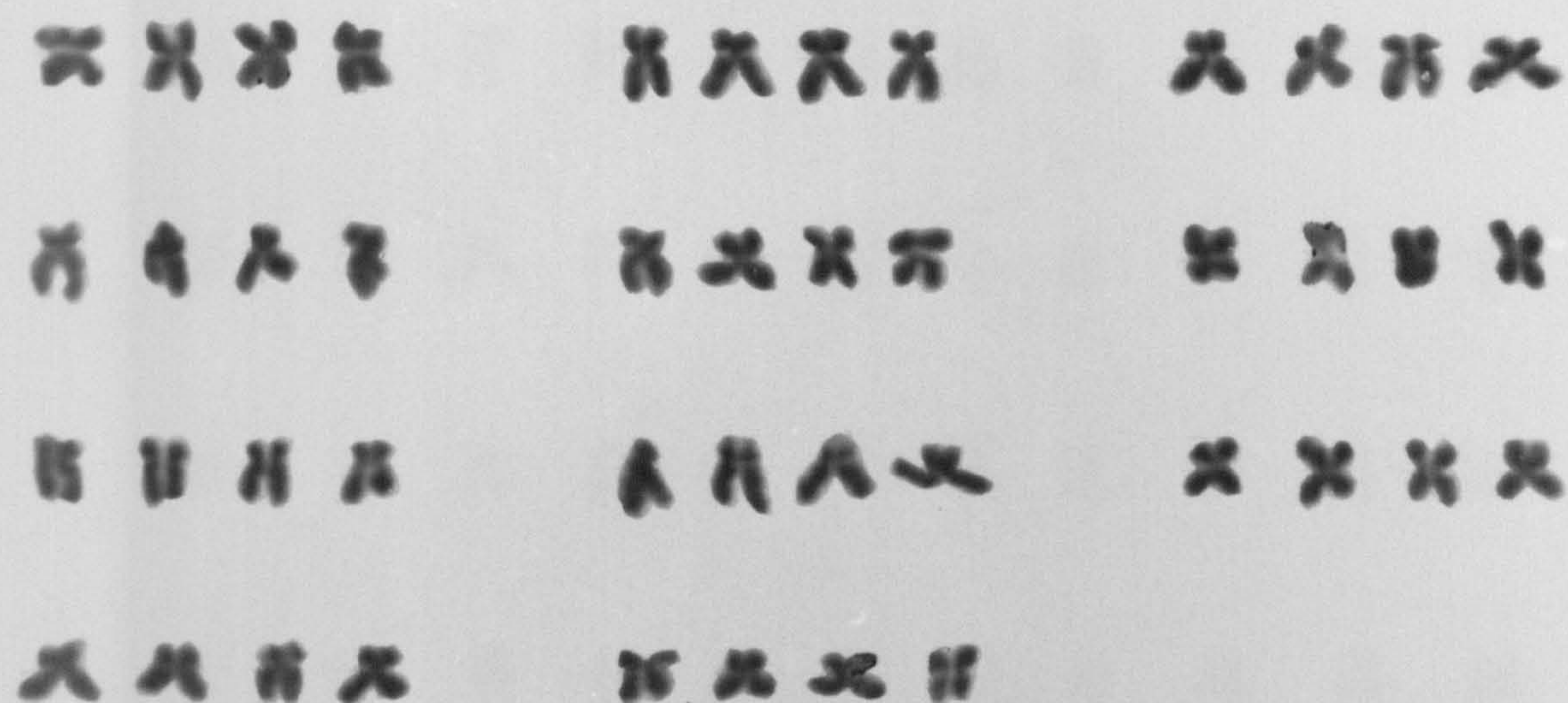


Figure 3.8

Karyotype and karyogram of *R. japonica* P134b $2n = 44$

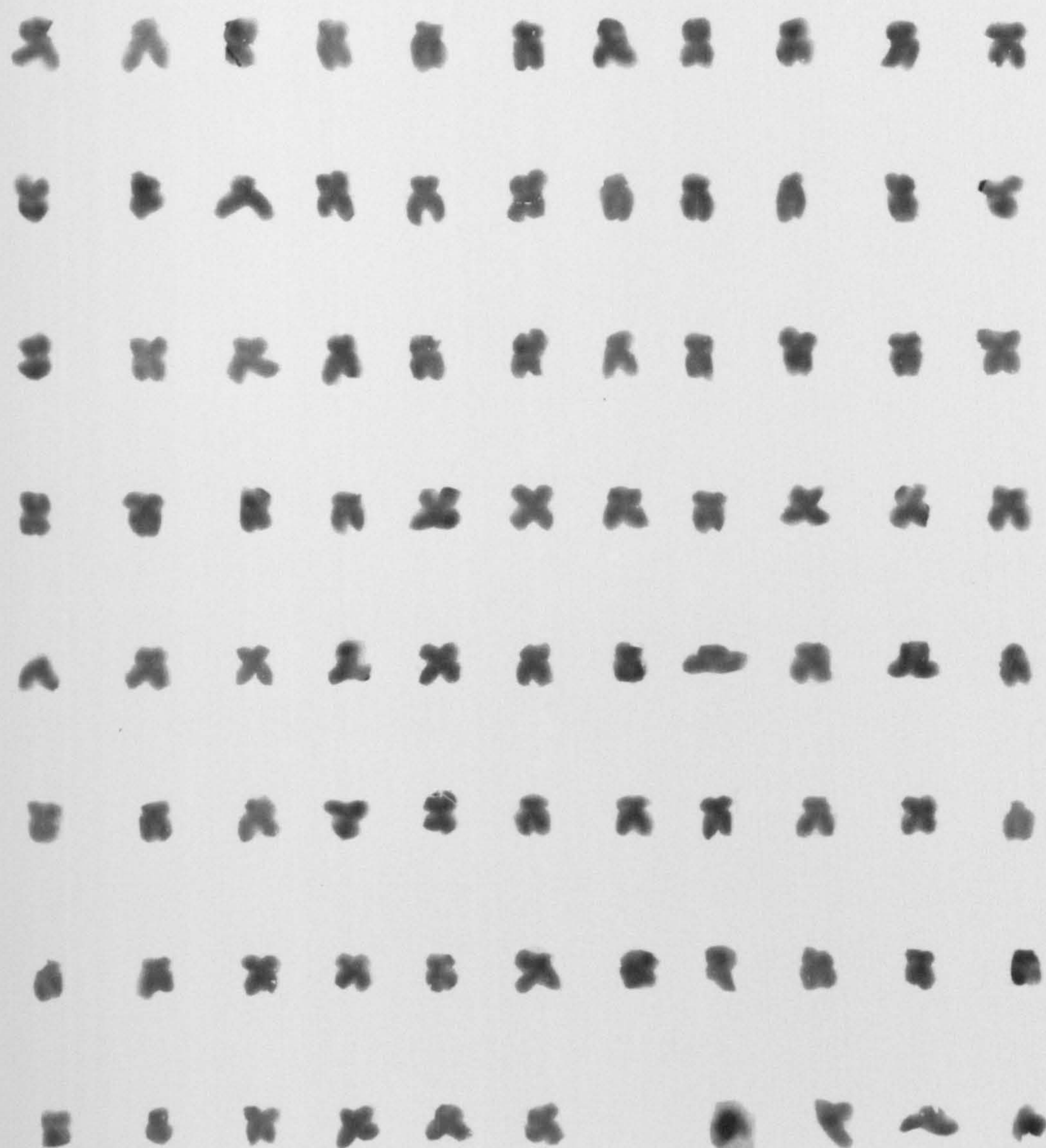


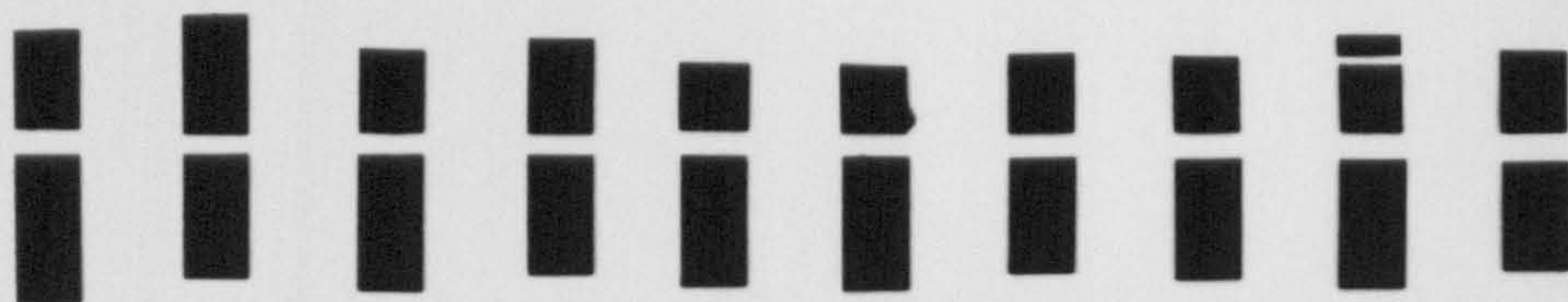
Figure 3.9

Chromosomes of *R. japonica* P105c arranged in order of decreasing size. Although this accession has 88 chromosomes one chromosome was missing from this cell.

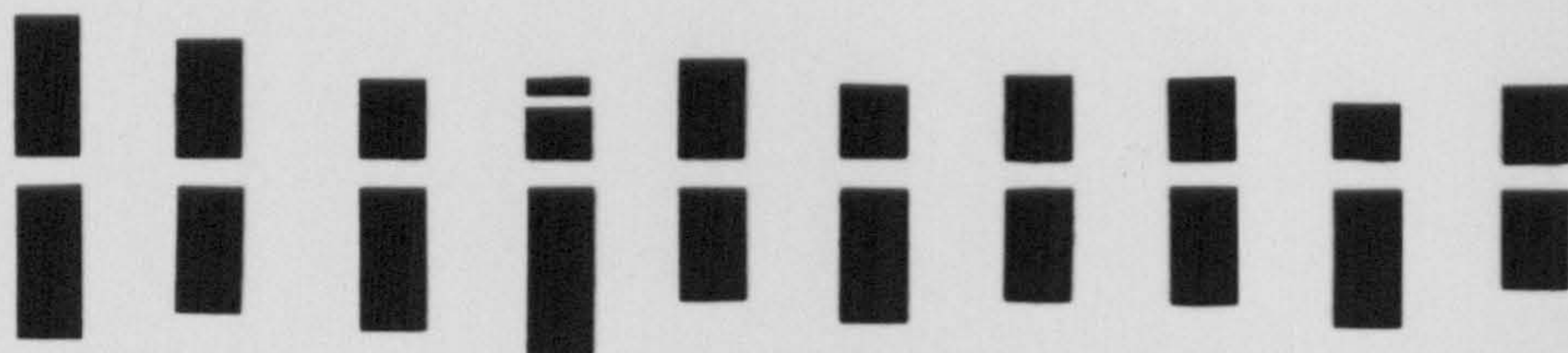
Figure 3. 10 2C diploid equivalent DNA corrected karyograms of eight Reynoutria and Fallopia taxa

- A *F. scandens* var. *dumetorum* P177
B *F. convolvulus* P150
C *R. japonica* var. *compacta* P99a
D *R. sachalinensis* P155

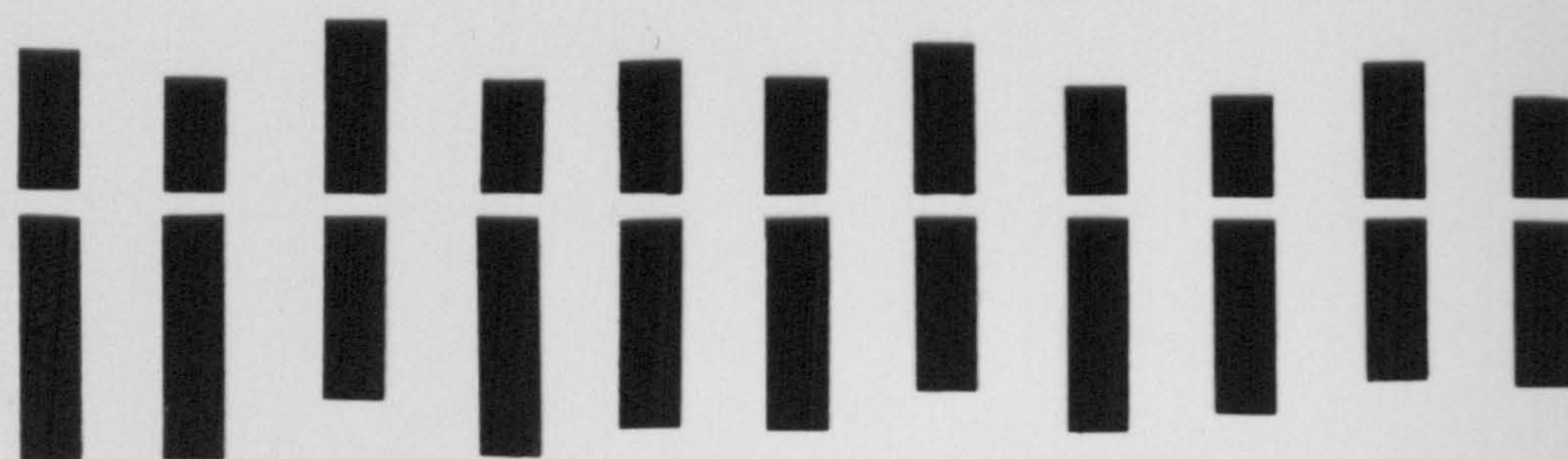
A



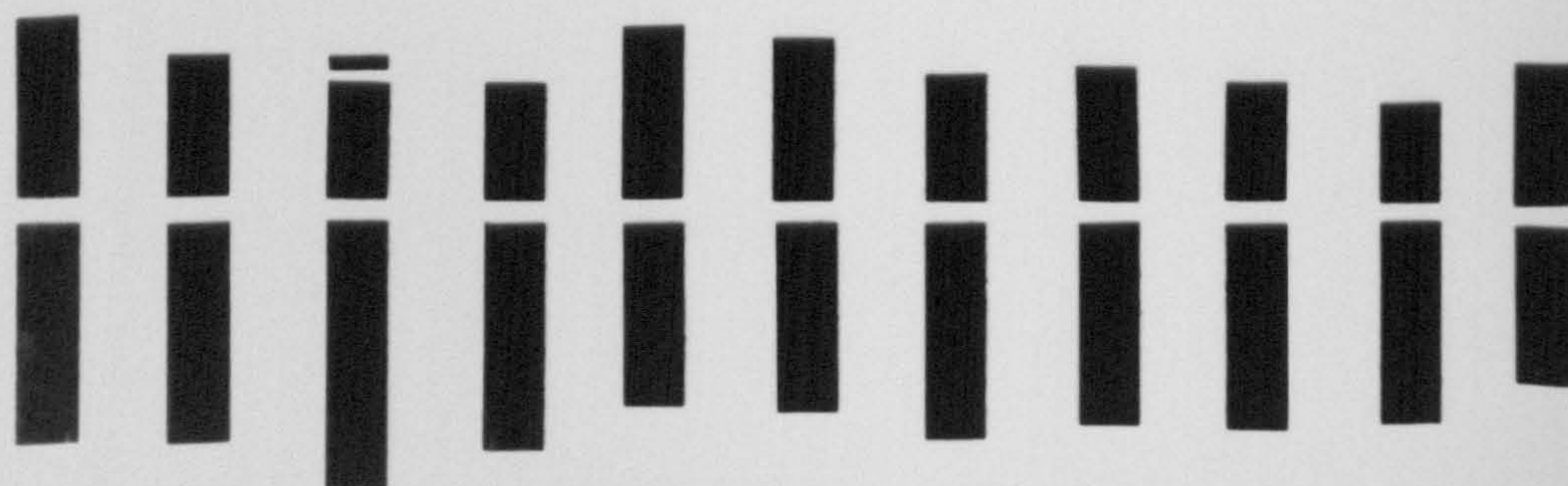
B



C

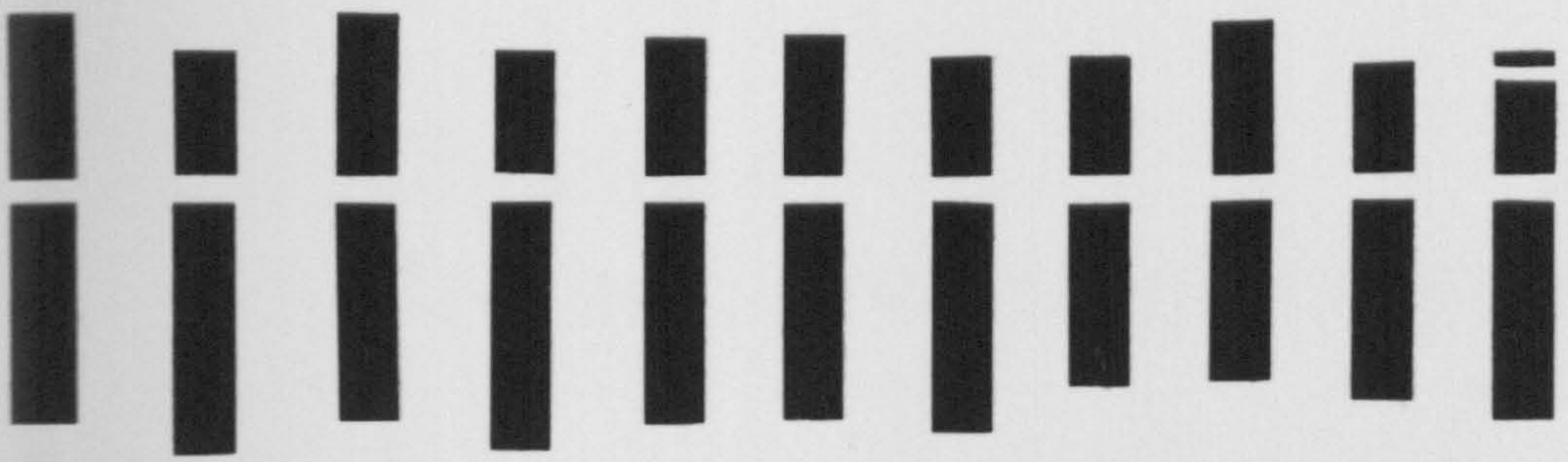


D

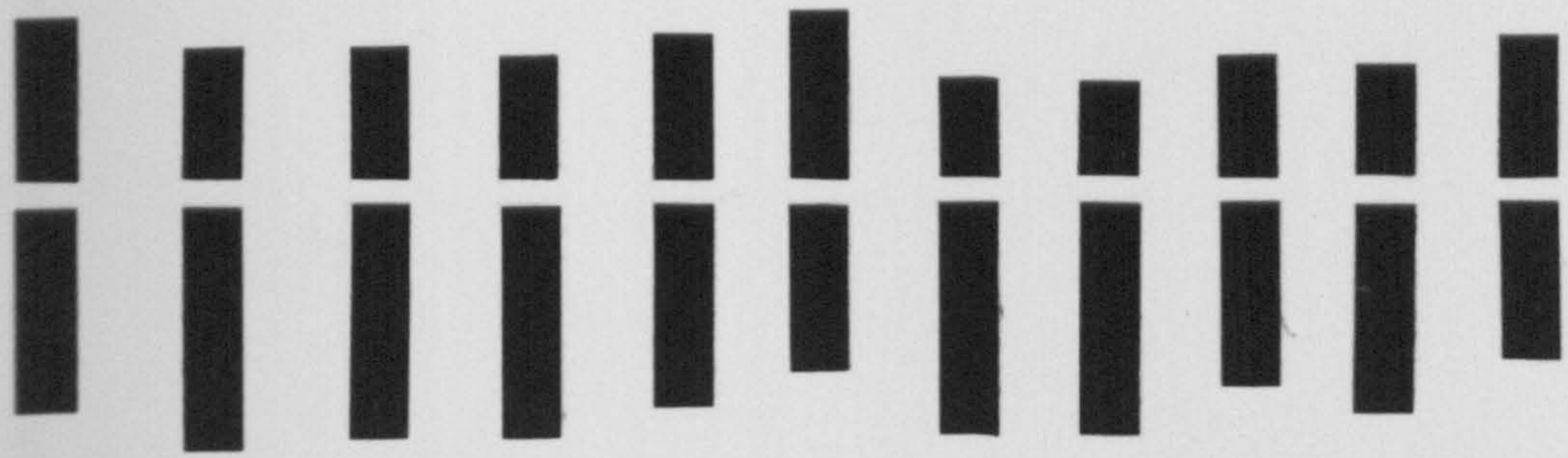


E *F. multiflora* P162
F *R. japonica* P134b
G *F. baldshuanica* P163
H *F. cilinodis* P148

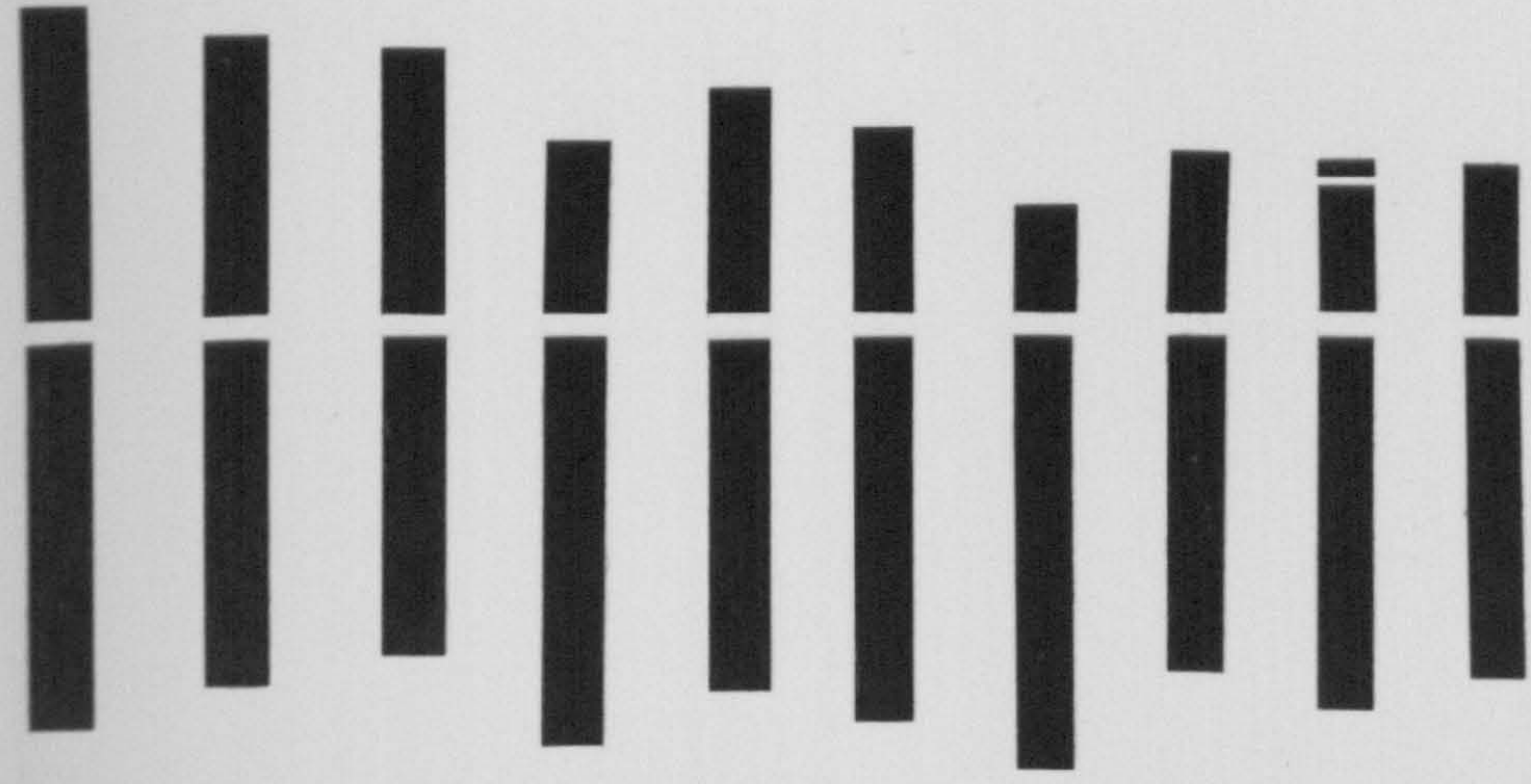
E



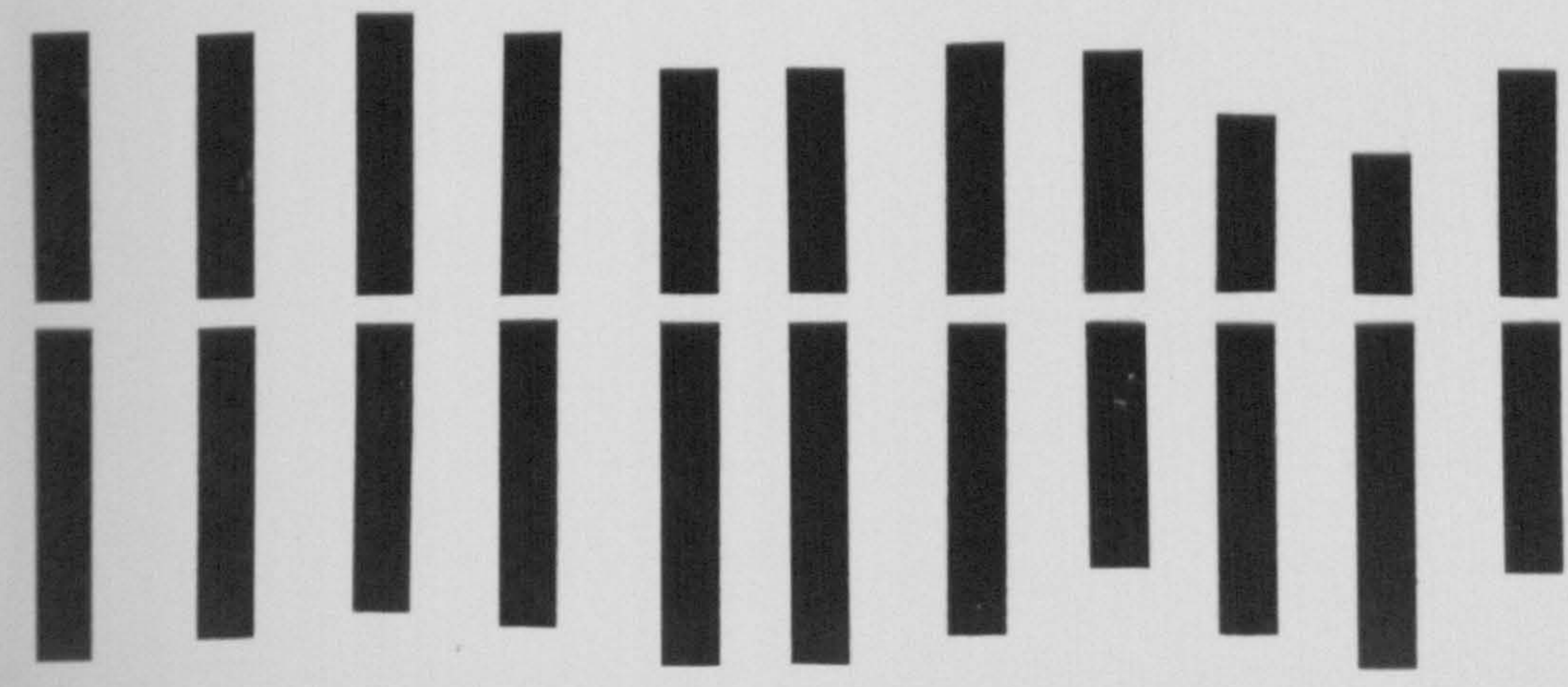
F



G



H



Chapter 4

Sex and breeding behaviour

4.1 INTRODUCTION

Plants or animals arriving in small numbers in regions far removed from their native habitat face a number of problems in coping with their new environment. Such founder populations occupy a precarious position. In the case of pairs of breeding birds (Mayr 1965) it is quite common for them to become extinct after a few years or decades despite apparent initial success. These founder populations inevitably begin with a rather uniform gene pool, though in time genetic variation can accumulate and compensate for the deleterious effects of inbreeding. Climate is another major factor, since most species from warmer climates cannot physically survive in the British Isles. In plants even fairly small differences in the length of the season can produce problems of seed-set for those plants which flower in the Autumn, (such as the Reynoutrias), particularly as the onset of winter here is rather unpredictable. The severity of the winter and its length will affect germination and early survival of any seedlings produced. In insect pollinated plants the appropriate pollinators might not be available, and even if they are, the low density of plants might mean that specialist pollinators such as honey bees ignore them as a source of nectar. The genetic constitution of the initial introductions is also an important factor, particularly in the case of garden plants where hybrid material might have been selected with a consequent reduction in fecundity. The lack of genetic variation resulting from a limited number of pioneer plants may have other consequences as well particularly in those

readily propagated vegetatively. In monoecious plants with a self-incompatibility mechanism there may be insufficient variation to allow seed set; whilst in dioecious taxa one sex may have been chosen on aesthetic grounds with obvious results.

In sexually reproducing plants success is generally related to the possession of an effective breeding system and reliable seed production. This is even more important when the invasion of new habitats is concerned. The Reynoutria taxa have had a spectacular success in colonizing parts of the British Isles (Conolly 1977), and the extent to which their breeding system has contributed to this success is one of the main concerns of this Chapter. In order to do this it is necessary to know something about the sex expression, population structure and the frequency and viability of seed produced in the British Reynoutria populations.

4.1.1 SEX EXPRESSION AND SEED SET IN REYNOUTRIA

What, if anything, may be gathered from the literature regarding the sex expression and breeding behaviour of these plants. The earliest descriptions of R. japonica are not very helpful since the presence of vestigial anthers in the male-sterile plants led to their being termed hermaphrodite. The illustration in Houttuyn (1777) is not detailed enough to distinguish the sex of the plant depicted though the Type Specimen from Geneva from which the drawing was presumably made is certainly male-sterile. The first clear illustration of a R. japonica flower is that of de Vriese (1849) and is clearly male-sterile. This is of particular interest since the plant came from Von Siebold's nursery at Leiden, the most probable source of the initial European introduction, and still probably the most commonly found clone in this country.

Hooker (1880a) described Reynoutria as dioecious, and in the case of R. japonica var. compacta he gave a detailed description of male-fertile and male-sterile flowers. R. japonica (1880b) was described as dioecious, but no mention was made of male-fertile plants in Britain; R. sachalinensis (1881) was listed as '?polygamous'. In all three cases it is the male-fertile plant that is illustrated. Ohwi (1965), in the Flora of Japan, and Webb (1964) in Flora Europea both listed Reynoutria taxa as dioecious. Conolly (1977), on the other hand, suggested that in R. sachalinensis male-fertile, male-sterile and hermaphrodite individuals may be found, recalling the polygamous condition noted by Hooker. She

also reported that a R. sachalinensis plant from Amroth (P68) had male-fertile, male-sterile and hermaphrodite flowers all on a single inflorescence. The observation at Leicester of apparently good seeds on a male-fertile plant of R. japonica var compacta (see Plate 4.1) also suggested that the sex expression of these taxa might be more complex than straight dioecy.

Conolly (1977) also commented on the rarity of male-fertile plants of R. japonica, and the relative imbalance of male to female clones in R. sachalinensis. Bailey and Conolly (1985) made a correlation between male-sterility and the octoploid chromosome level in R. japonica. Conolly (1977) brought together the existing knowledge and observations regarding seed production in Britain; there had been few reports of it and no information at all regarding its viability, germination and survival in the British climate. Various reasons were put forward to account for this low incidence of seed-set, including early cut back by frost and the paucity of suitable pollen, especially in the case of the octoploid R. japonica which appears to be an all male-sterile population. She concluded that seed-set and subsequent seedling production was probably not important in the dispersion of Reynoutria plants in this country, vegetative reproduction from rhizome fragments dispersed by Man's activities being the principal mode of spread.

This is clearly an interesting problem, and one which would benefit from evidence of a more quantitative nature.

4.1.2 POLLINATION AND SEEDLING ESTABLISHMENT OF R. JAPONICA IN JAPAN

Not a great deal has been published on the reproductive biology of R. japonica and that which has, has been rather incidental to the author's main theme, and in any case only refers to Japan. Maruta (1983), in what is essentially an ecological study, gave an interesting account of seedling survival and establishment of R. japonica in the volcanic gravel of Mount Fuji. He compared survival of seedlings at 1400m a.s.l. and 2500m a.s.l. At 2,500m a.s.l. the plant grows as a pioneer species and seed production is obviously of great importance, but unfortunately there is no information about the ecology of these plants when growing in lowlands or cultivated areas, where it may be inferred that seed set and dispersal might not be so crucial. Regrettably Maruta gave no indication of the size of the plants, but, since at 2,500m a.s.l. the growing season is only 70 days, one would anticipate that rather small if not dwarf plants such as R. japonica var. compacta are involved.

At 1400m a.s.l. the seed germinates in late May, and if it is to survive its first winter (with a minimum temperature of -14°C) it must attain a dry weight of at least 10mg, otherwise it is unable to suberize the rhizome or produce the two perennating buds. From his account, recruitment of new plants from seed seems to be very much the norm, with populations said to consist of current year seedlings, small plants of various ages and stands of adult plants. When growing above the tree line at 2500m a.s.l. conditions are

extremely harsh, and only 3% of the germinated seedlings survive the winter. Plants capable of surviving such rigours ought not to find an English winter too difficult providing that they could stand wet (rather than dry) cold conditions. It may be that reasons other than climatic are responsible for the lack of reports of seedling establishment in Britain.

Useful information about the pollinators of R. japonica in its native habitat in Japan is supplied by Tanaka (1966). He made observations of both male-fertile and male-sterile stands, listed all insect visitors, their reason for visiting (nectar or pollen) and whether they were effective pollinators. The value of this work is somewhat diminished by his use of trivial names for the insects, this proved to be beyond the capabilities of my Japanese translator. Fortunately some were illustrated and I was able to piece together the following. Honey bees and rather surprisingly wasps were listed as good pollinators and were attracted by both the abundant nectar (which is produced by both sexes) and pollen. Twelve species of Diptera were recorded as visitors, but only three (bee mimics and house flies from the illustrations) were involved in pollination. Butterflies and moths were also attracted to these flowers but were not regarded as effective pollinators as their legs did not touch the inside of the flowers. One species of beetle was also reported as a pollinator.

See also

and when

See also

4.1.3 THE PROBLEM OF SEX EXPRESSION AND SEED PRODUCTION

From the preceding accounts it can be seen that there is no shortage of anecdotal information regarding these plants, but interesting as such reports are there is now a real need for a more rigorous quantitative approach to the problem. A number of questions need to be answered. Firstly can these plants actually produce viable seed in Britain, can it germinate and can it survive long enough to become established in our climate? If the isolated male-sterile octoploids can and do produce seed, what is the parentage of such seed? Is it apomictic? Such considerations are important since the nearest male-fertile Reynoutria plants may be ten or twenty miles away. Secondly, what is the precise nature of the balance of sex expression of these plants in Britain? Can female plants become male or vice versa, and are R. sachalinensis plants polygamous as has been suggested? The usual assessment of female fertility is by seed-set, yet if self-ⁱⁿ-compatibility mechanisms operate in these plants an hermaphrodite growing by itself would not be expected to produce seed, and would on this basis be wrongly regarded as female-sterile.

These plants are not ideal candidates for the student of reproductive biology, since they need to be quite large before they will flower, and their rhizomatous habit makes them most unwelcome at Botanic Gardens. Their late flowering can also cause problems as in fact happened in 1986 when a cold late summer and early autumn caused the flowers often to drop without opening, effectively

terminating a programme of controlled pollination and observations of seed-set. The problem of where to grow them was solved by rather a novel means at Leicester, when some large tiled fish-tanks outside the Biological Sciences building were declared redundant and filled with soil! These made ideal Reynoutria containment vessels and came complete with water supply. The Autumn of 1987 gave me a unique opportunity to estimate the seed production of a large collection, grown in comparable conditions, of mature cytologically-known accessions in the presence of large amounts of compatible pollen.

4.2 RESULTS

4.2.1 SEX EXPRESSION IN REYNOUTRIA

The Reynoutria collection at Leicester has been built up over many years and consists of mature examples of clones found in Britain supplemented by material grown from seed from Japan and China, and encompasses the whole range of sex expression found in Britain, being particularly strong on the male-fertile clones. With regards to terminology I shall be using the terms male-fertile and male-sterile; no pollen has yet been found in plants designated male-sterile, and male-fertile covers hermaphrodite and female-sterile conditions. Figure 4.1 shows the proportion of male-fertile and male-sterile clones from Britain arranged according to their ploidy level. This is not, however, a random survey since it includes virtually every male-fertile plant that we have been able to examine. In the country as a whole it must be said that the male-sterile R. sachalinensis is much more common than is indicated in the graph. It will also be noted that the octoploid R. japonica is represented only by male-sterile plants but even so is not truly representative of the vast predominance of this clone in Britain. An example of the low frequency of male-fertile Reynoutria plants may be gained from the fact that there are no known male-fertile plants growing in the wild in Leicestershire (2 were known but have both been eradicated), in contrast to thousands of what on morphological grounds may be attributed to the male-sterile octoploid category. In R. japonica var. compacta the sexes are more evenly balanced, though four

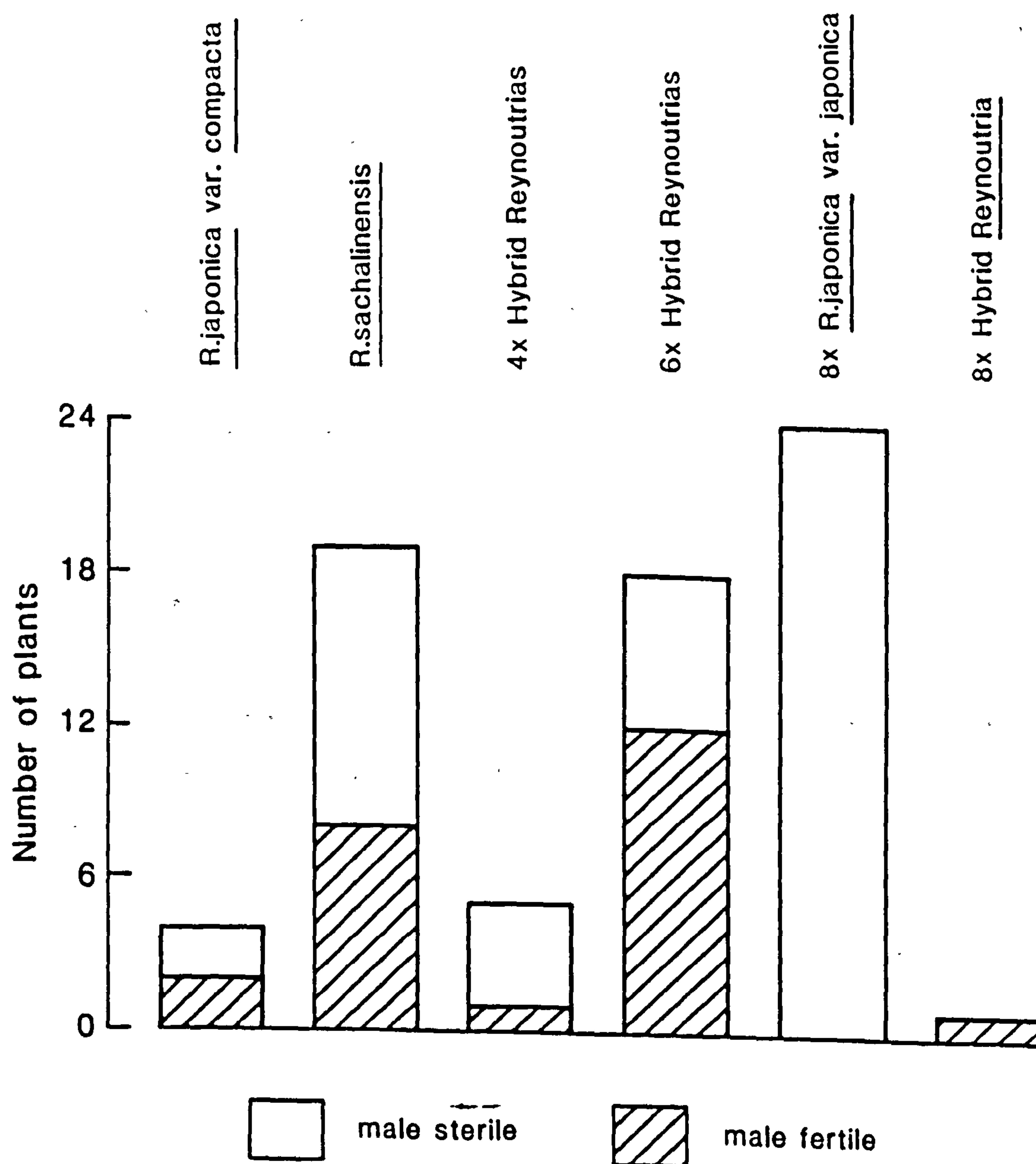


Figure 4. 1 Proportion of male - sterile and male -fertile *Reynoutria* plants from the British Isles which have been cytologically investigated

plants can hardly be called a representative sample. This is, however, a reflection of the relative rarity of this variety in Britain. There is just a hint of an overabundance of males at the hexaploid level, although this might be a reflection on our sampling technique since all male-fertile plants noticed were sampled whilst male-sterile ones were not. It is not impossible, should it be proved that male-fertiles are more common at the hexaploid level, that this intermediate ploidy level has had some effect on the sex-determining mechanism.

Having determined the distribution and frequency of male-fertile and male-sterile plants the next stage was a detailed examination of the sex organs of flowers from the plants of these two groups. It will be recalled that the basic floral structure of Reynoutria flowers is a five tepaled perianth with a trigonous gynoecium, short style and trifid fimbriate stigma surrounded by 8 anthers. In male-sterile plants the anthers are small flattened, empty and included within the perianth, whereas the male-fertile flowers have large full anthers well exerted from the perianth, and display varying degrees of stigmatic development. Between 15 and 40 flowers were collected from 19 clones (all but one growing in our collection). Newly opened flowers were chosen, since fertilization and other developmental changes would affect the reliability and comparability of the measurements. Measurements were made by dissecting the flowers under a stereo-microscope fitted with an eye-piece graticule and are tabulated in Table 4.1. Stigma development is expressed developmentally rather than

Accession	Sex	Gynoecium length (mm)	Style + stigma length mm	Stigma develop- ment	Ovary width (mm)	Filament length (mm)	undehisced anther length (mm)	Comments
R. <u>jap. v. comp.</u> P173	male-fertile	1.14	0.26	+ to ++	-	-	-	hermaphrodite
R. <u>jap v. comp</u> P99	male-fertile	0.79	0.2	-	0.3	1.9	0.57	
R. <u>sachalinensis</u> P57	male-sterile	1.17	0.3	++ to +++	0.65	0.57	0.25	
R. <u>sachalinensis</u> P68	male-fertile	1.6	0.45	+ to +++	0.68	1.8	0.7	hermaphrodite
R. <u>sachalinensis</u> P55	male-fertile	1.9	0.35	+ to ++	0.76	1.8	0.81	hermaphrodite
R. <u>sachalinensis</u> P62	male-fertile	1.44	0.45	+ to ++	0.54	2.1	0.92	hermaphrodite
R. <u>japonica 4x</u> P114	male-fertile	1.96	0.39	+ to +++	0.92	0.99	0.94	hermaphrodite
4x <u>Reynoutria</u> P13	male-fertile	1.16	0.25	- to ±	0.54	1.14	0.78	hermaphrodite
4x <u>Reynoutria</u> P79c	male-fertile	0.71	0.14	-	0.36	2.59	0.92	
6x <u>Reynoutria</u> P52	male-sterile	1.48	0.5	++ to +++	0.70	0.47	0.39	
6x <u>Reynoutria</u> P119	male-fertile	0.9	0.15	-	0.43	2.04	0.75	
6x <u>Reynoutria</u> P45	male-fertile	1.19	0.34	- to ++	0.5	1.7	0.6	hermaphrodite
6x <u>Reynoutria</u> P75c	male-sterile	1.19	0.36	+ to ++	0.56	-	-	
6x <u>Reynoutria</u> P75d	male-fertile	1.3	0.39	- to ±	0.61	1.75	0.81	hermaphrodite
6x <u>Reynoutria</u> P76b	male-fertile	0.72	0.15	-	0.38	2.6	0.97	
R. <u>jap v. jap. 8x</u>	male-sterile	1.6	0.42	+ to ++	0.7	0.5	0.31	
8x <u>Reynoutria</u> P51b	male-fertile	2.04	0.58	+ to +++	0.8	2.7	0.99	hermaphrodite
R. <u>jap. v. comp.</u> P2b	male-sterile	1.63	-	+ to ++	-	0.46	-	
6x <u>Reynoutria</u> P31	male-sterile	1.5	-	+++	-	0.48	-	

TABLE 4.1 STIGMA DEVELOPMENT, OVARY SIZE AND STAMEN LENGTH OF MALE-FERTILE AND MALE-STERILE REYNOUTRIA

quantitatively, and it may be taken that anything recorded as + and upwards contains some receptive stigmatal surfaces. An examination of this table and the associated graph Figure 4.2 shows the plants to divide into three groups. Group one (shown as filled) constitutes the male-sterile and is characterized by a gynoeceum in excess of 1.1mm, a well developed style and a stigma in excess of 0.3mm, with small empty anthers on short filaments. The second group may be termed female-sterile since they have short, poorly developed gynoecea lacking altogether in stigmatal development, and large well filled anthers borne on long exserted filaments. The third group is intermediate, possessing the large ovaries with well developed stigmas in conjunction with large filled anthers borne on long filaments. This third group are then morphologically hermaphrodites, but the question of whether they can function as hermaphrodites is addressed later. The three Reynoutria flower morphs are illustrated diagrammatically in Figure 4.3. It should be noted that these drawings do not represent the relative exsertion of the male and female parts, merely their relative dimensions. (Since total ovary length is used it includes that part which is below the stamen bases, but the filament measurements are of the free part only, the adnate bases being ignored).

Whilst there are no reliable reports of male-sterile Reynoutria ever producing pollen, there are reports of male-sterile flowers on male-fertile plants. Some of these result from late season observations of a known male-fertile plant where the presence of seed is taken to denote a female

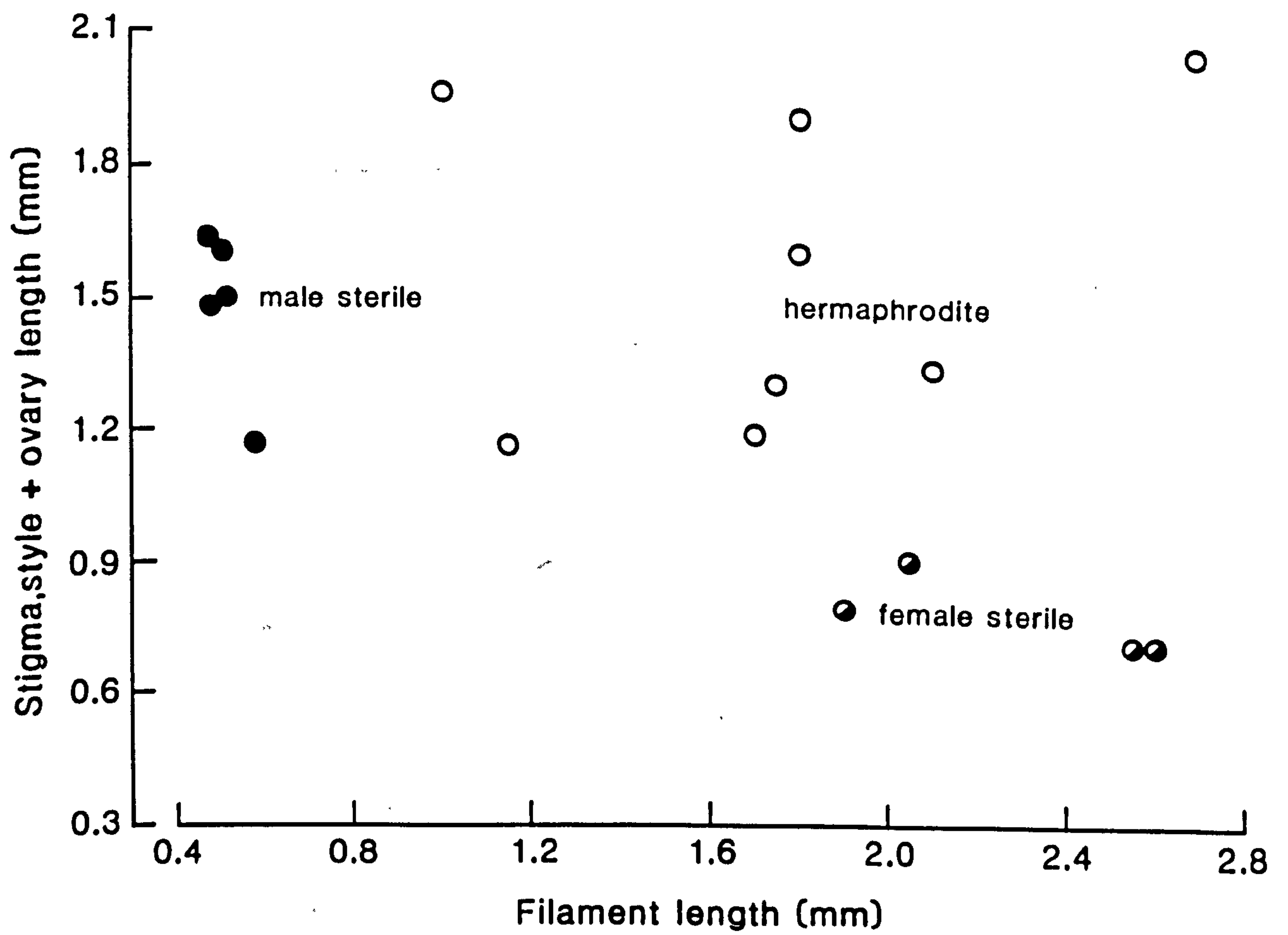
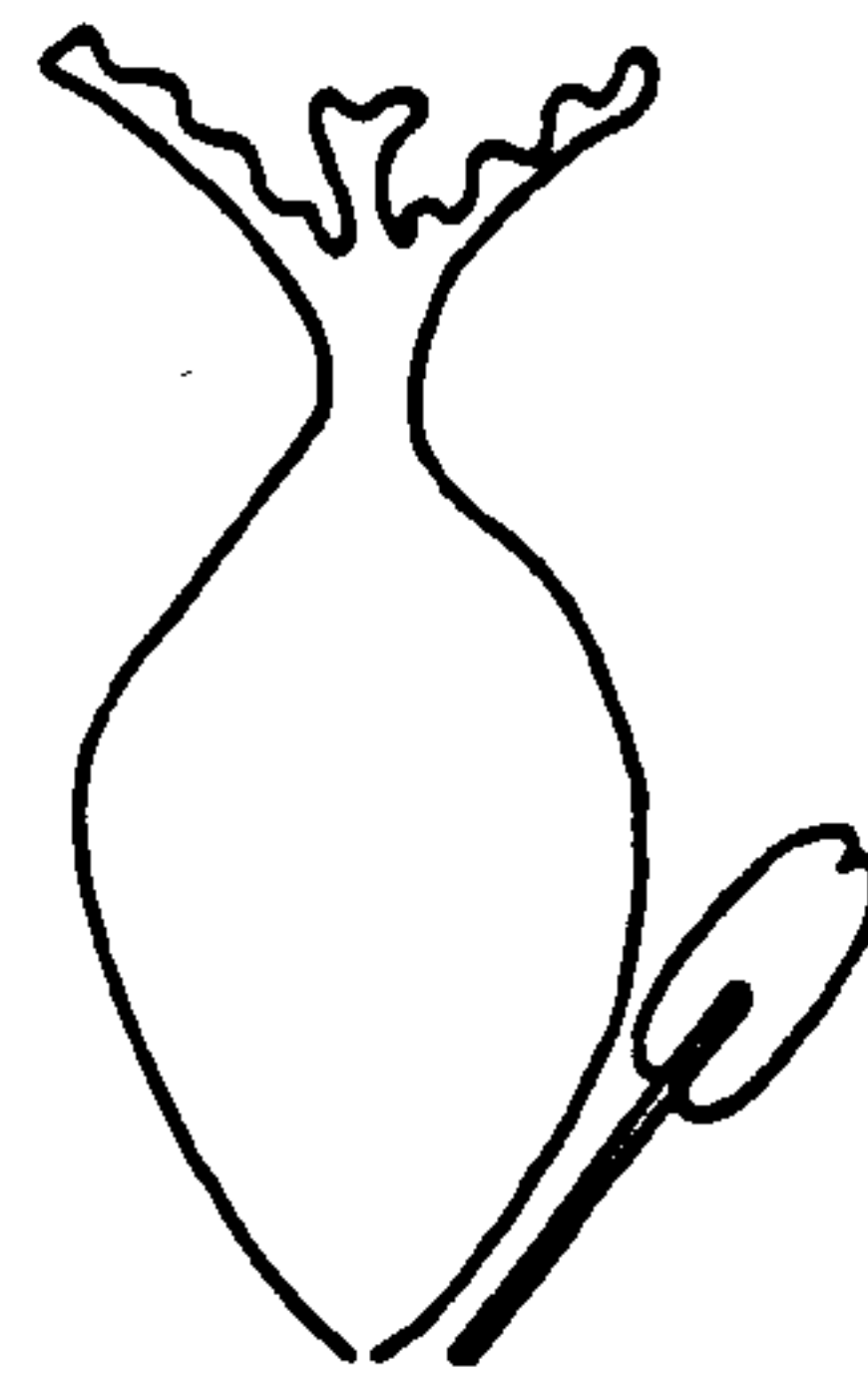
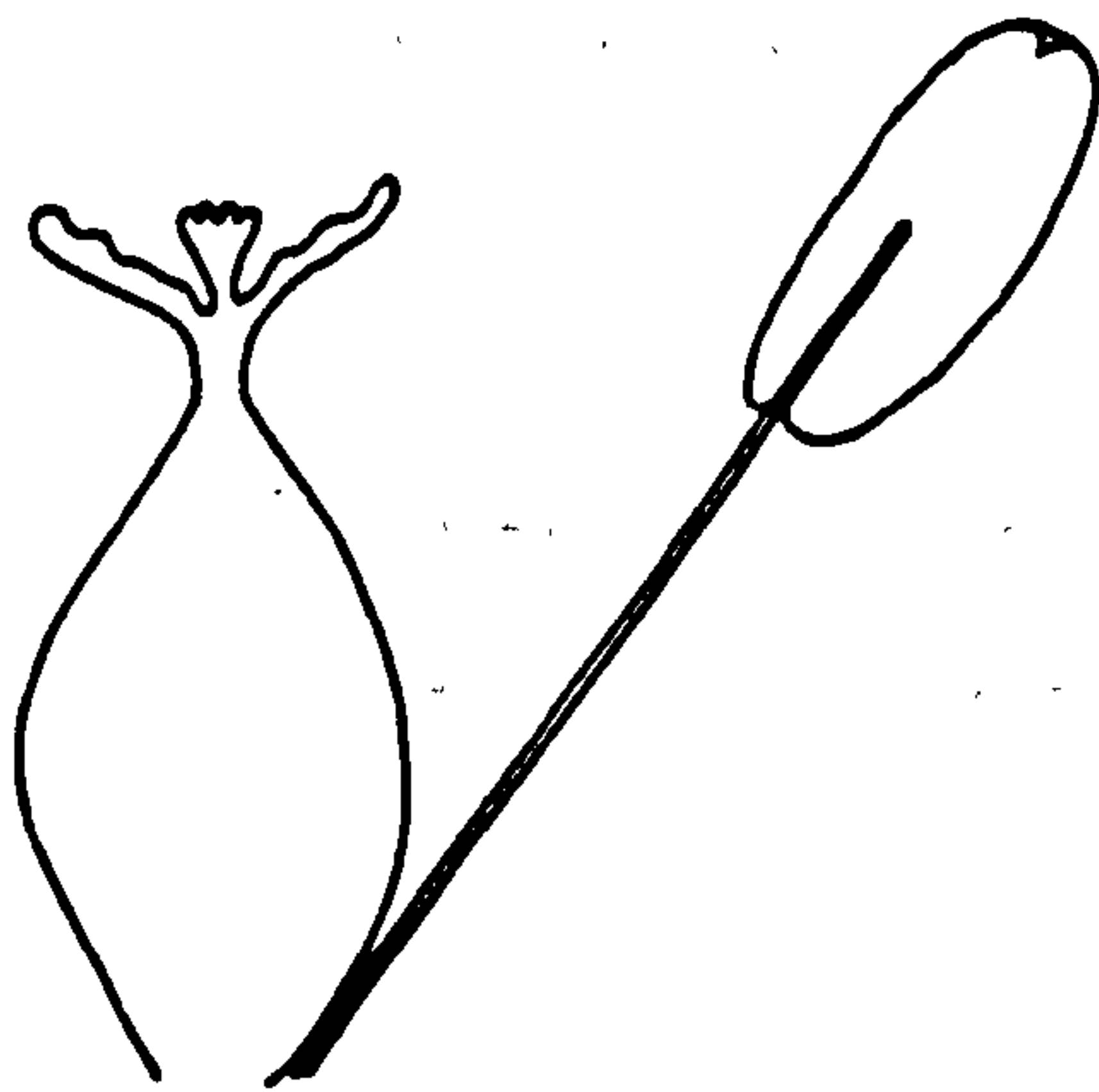


Figure 4.2 Total gynoecium length X anther filament length in *Reynoutria* clones

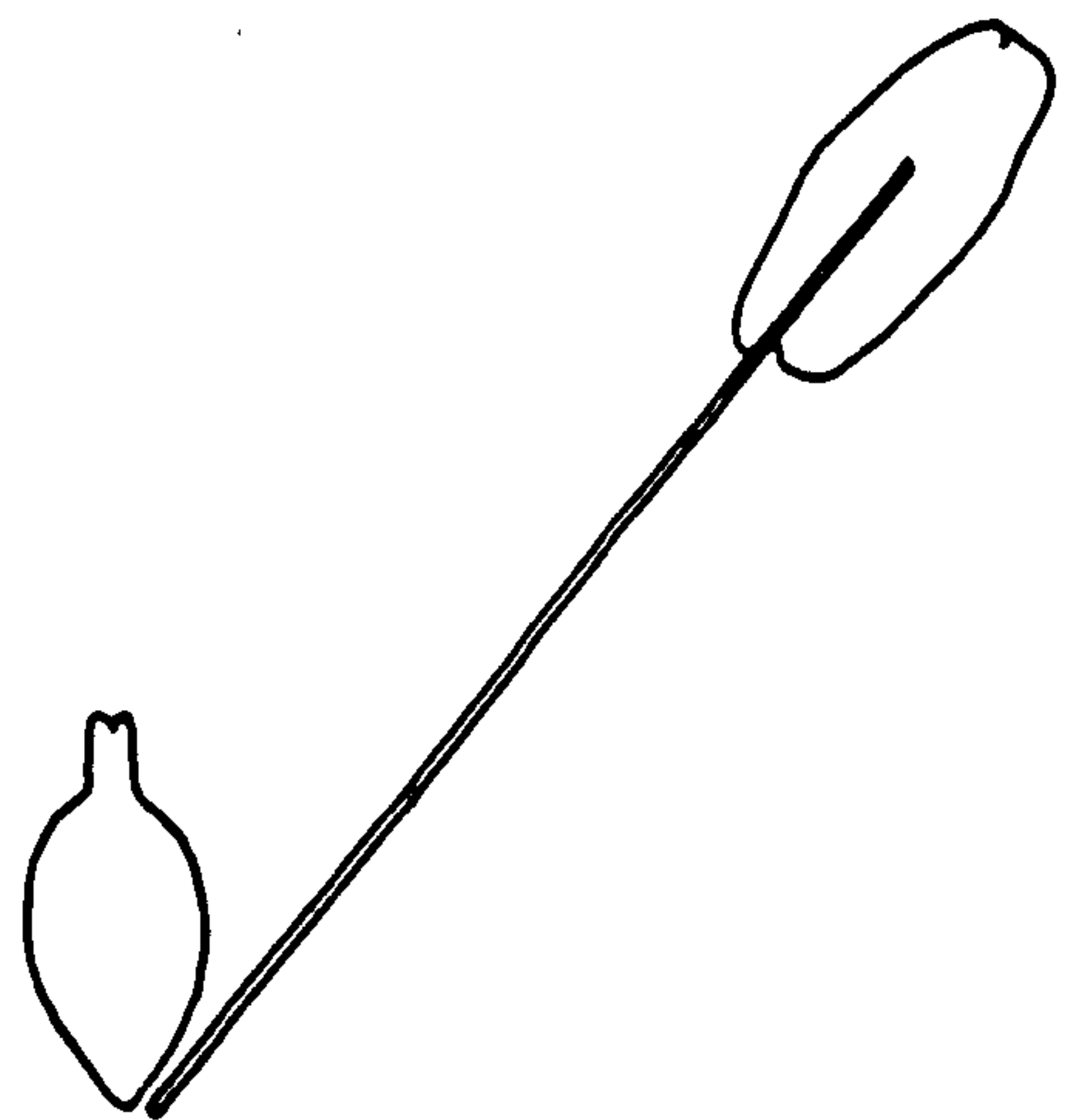
1 mm



1 male-sterile



2 hermaphrodite



3 female-sterile

Figure 4.3 The 3 flowering morphs of *Reynoutria*

flower. Male-fertile plants, particularly late in the season, can show variable amounts of stamen and stigma development. Figure 4.4 graphically shows one such plant, hexaploid Reynoutria P45, where the majority of the flowers contain small ovaries without any stigmatal development, with a significant minority having large ovaries and well developed stigmas. Regarding anther development, one sometimes finds flowers in which only 1 or 2 of the anthers are clearly fertile, and in which stigma development is somewhat enhanced. Other flowers from male-fertile plants have been found with brown sterile anthers, suggesting that that part of flower is dead rather than abortive as the case in normal male-sterile flowers. This combination of late season, variability or death of the male organs is strongly suggestive of some climatic effect on the developing anthers. A particularly cold autumn day may for instance inhibit or abort meiosis in those anthers on the outside of the bud, a more severe cold shock perhaps killing off all the anthers. Plates 4.2c,d show the range of sex expression of an R. sachalinensis plant from Osgathorpe which is unfortunately no longer in our collection. It may be seen that not all the anthers with short filaments are necessarily sterile, since some appear to be of a good size; note also the well developed stigmas.

Hence the evidence indicates that we are dealing with gynodioecious populations in which the hermaphrodite plants exhibit some climatically induced lability in male-fertility in late autumn.

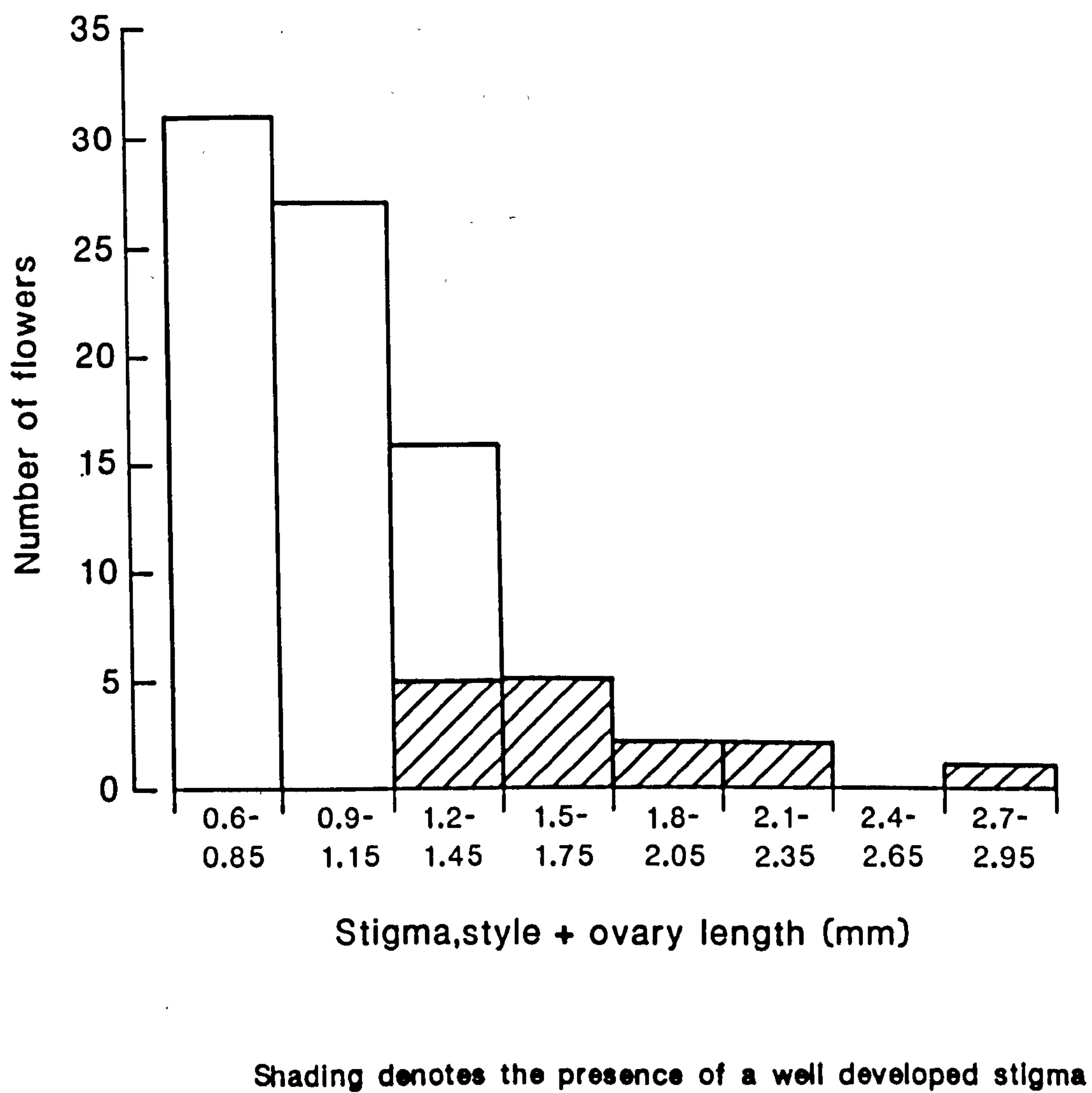


Figure 4.4 Distribution of the extent and degree of gynoecium development in a 6x *Reynoutria* (P45)

4.2.2 SEX EXPRESSION IN FALLOPIA

Those taxa that are diploid or have diploid cytotypes appear to be functionally hermaphrodite. F. convolvulus. F. dumetorum and F. cilinodis all have hermaphrodite flowers and are self compatible - F. convolvulus almost to the point of cleistogamy. F. multiflora is something of a mystery since I only know its flowers from herbarium specimens and descriptions. Although sometimes listed as dioecious (Ohwi 1965), the herbarium specimens seem to contain hermaphrodite flowers. F. baldschuanica is the only taxon that I have investigated in detail; it is hermaphrodite but with a very strong self incompatibility mechanism (see Plate 4.4c, d), which probably accounts for its extremely poor seed-set in this country.

4.2.3 FECUNDITY OF THE HERMAPHRODITES

It is one thing to demonstrate that a flower is morphologically hermaphrodite, but quite another to show that it in fact functions in that way. This problem has been approached from two directions: by examination of seed-set in hermaphrodites both in the wild and in the collection at Leicester; and by the in vitro pollination of the stigmas of hermaphrodite plants, both with their own pollen and pollen from different clones. Since no male-fertile octoploid R. japonica is known from the British Isles this work has centred mainly on R. sachalinensis and the 4x and 6x Reynoutria. Fertile seed is produced on occasion by hermaphrodite R. sachalinensis in the wild but not in any appreciable amounts. Visits to the extensive stands of hermaphrodite R. sachalinensis at Amroth produced, after a diligent search of several hundred stems, a total of 44 seeds in 1985 and 112 in 1986. Similarly an earlier visit to the R. sachalinensis at Brithdir produced only 12 seeds. Whilst it could be argued that some seed had blown off prior to my visits, it is generally my experience that the nutlet is shed before the persistent winged perianth (whether or not assisted by seed-eating birds) and there were not significant numbers of winged perianths without nutlets on these visits. Seed produced by male-fertile plants tends to be of a rather different shape from that produced by male-sterile plants (see Plate 4.2a) often lacking the wings or with irregular wing development. It could then be easily dismissed as empty or overlooked without a careful dissection of fruiting perianths. Plate

4.2b shows fruits produced by male-fertile and male-sterile 4x Reynoutria of the same genetic constitution. Fruits from male-fertile plants lack the broad regular wings of the male-sterile fruits, their smaller size and irregular wings often belying the presence of fertile seed. Since an hermaphrodite R. sachalinensis plant grown in the collection at Leicester produced 2,780 fruits on a single stem (admittedly much fewer than male-sterile plants) it would suggest that the reason for low seed-set on hermaphrodites growing in the countryside is lack of compatible pollen. In order to investigate this possibility an examination was made of the behaviour of selfed and non-selfed pollen on the stigmas of the hermaphrodite R. sachalinensis plants (for technique see Chapter 5). Results of this are shown in Table 4.2. There was little pollen germination with selfed stigmas, although good germination was observed when compatible pollen was used. Plate 4.5c shows an hermaphrodite R. sachalinensis stigma with compatible pollen indicating good germination and growth along the style. Plate 4.5d shows a stigma from the same plant with selfed pollen; there has been low germination and tubes produced are meandering around and have irregularly shaped tips indicating a stigmatic incompatibility reaction.

Batches of seed from male-fertile R. sachalinensis (P55) growing at Leicester gave 33 and 57% germination respectively with a simple continuous light and temperature regime. I did not risk natural germination of the valuable seed collected from Amroth and so germinated it by means of embryo culture (methods in Chapter 5). Even seeds deficient

Taxon	No. stigmas examined	Pollen attachment	Pollen Germination	Pollen Penetration	Incompatibility reaction	Comments
<u>F. dumetorum</u> P177	10	+++	+++	+++	-	Self fertile; regular seed-set
<u>F. baldschuanica</u> P174	30	+ to ++	+ to ++	+ to +	++	Tubes terminate in swellings
<u>F. baldschuanica</u> P163	25	++ to +++	+ to +++	+++	+++	Tubes do not grow beyond stigma
<u>R. sachalinensis</u> P55	10	+ to ++	-	-		Stigmas all well developed
<u>R. sachalinensis</u> P62	20	±	±	±		
<u>R. sachalinensis</u> P68	14	+	+	±	±	incompatibility reaction, but a few good tubes
<u>R. jap.v.comp.</u> P173	33	-	-	-		Stigmas not fully mature
<u>R. jap.v.comp.</u> P99c	7	+	+	±	±	
<u>R. jap.v.jap.4x</u> P114	7	±	-	-		
<u>R. jap.v.comp.</u>	5	++	+	-		Tubes irregular
<u>R. sach. (art.)</u> P78a						
Put. <u>R. jap.</u>						
x <u>R. sach. 8x</u> P50	10	±	-	-		Stigmas mature
Put. <u>R. jap x</u>						
<u>R. sach. 8x</u> P51B	20	±	±	±		

TABLE 4.2 POLLEN GERMINATION AND GROWTH ON SELFED MALE-FERTILE TAXA.

in endosperm had well-formed embryos which germinated readily, and 11 out of the 12 seedlings examined cytologically were recognisably R. sachalinensis with 44 chromosomes. This would point to the occasional breakdown of the self-incompatibility barriers resulting in the extremely rare fruit formation in the absence of normally compatible pollen.

There still remains the unanswered question of how plants categorized as female-sterile still manage to produce the occasional good fruit (see Plate 4.1) nor are reports of a polygamous condition in R. sachalinensis explained. The ideal solution to this would be to examine the development of individual flowers over a period of time in order to see whether and to what extent temporal or seasonal changes in the sex expression of individual flowers or clones are occurring. It is, however, very difficult in practise to map and locate individual flowers on a stem, especially when growing outdoors. Bringing the plants inside helps, but they are then shielded from some of the environmental variables that may be responsible for such changes. The other major difficulty is the extremely low frequency of such changes and consequently the vast number of flowers that must be monitored. The female-sterile plant P79C, for example, produced 5 seeds from an estimated 40,000 flowers. And although the male-fertile R. sachalinensis produced a seed-set of 5% the proportion of seeds produced by male-sterile flowers as opposed to hermaphrodite flowers is probably very small.

One indirect way of assessing such changes is by an examination of the fruiting perianths of seeds produced by male-fertile clones for the remains of anthers, so that they can be assessed as male-fertile or male-sterile. This is a fairly reliable means of establishing the sex of the flower that produced the seed. I have examined the fruiting perianths of fruits from male-fertile plants from a number of localities, the results of which are shown in Table 4.3. These demonstrate quite clearly that, whilst there are some male-sterile flowers on these plants and they can in fact produce seed, they are not a very significant source of seed, producing on average only 10% of fruits examined.

Taxon	Collection No.	Male-fertile Seed	Male-sterile Seed	% Male-sterile	Year	Situation
<u>R. sachalinensis</u>	P67	35	5	12.5	1984	wild
<u>R. sachalinensis</u>	P67	23	1	4.2	1985	wild
<u>R. sachalinensis</u>	P68	3	1	25	1984	wild
<u>R. sachalinensis</u>	P68	89	1	1.1	1985	wild
<u>R. sachalinensis</u>	P55	32	10	23.8	1981	cult.
<u>R. sachalinensis</u>	P62	33	-	0	1985	cult.
<u>R. sachalinensis</u>	P63	27	4	12.9	1985	cult.
<u>R. japonica</u> X <u>R. sachalinensis</u> (6x)	P32	38	5	11.6	1985	cult.
<u>R. japonica</u> X <u>R. sachalinensis</u> (8x)	P5Ib	36	0	0	1985	cult.
<u>R. sachalinensis</u>	P54	11	1	8.3	1983	wild
mean percentage male-sterile				9.94		

TABLE 4.3 SEED PRODUCTION BY MALE-FERTILE AND MALE-STERILE FLOWERS ON MALE-FERTILE REYNOUITRIA PLANTS (AS EVIDENCED FROM EXAMINATION OF THE FRUITING PERIANTHS)

4.2.4 QUANTITATIVE ESTIMATION OF SEED SET IN REYNOUTRIA

The autumn of 1987 presented a unique opportunity for an investigation into the seed-set of Reynoutria clones at Leicester; unique because the plants were all mature and growing well, a great diversity of pollen was available and weather conditions had proved conducive to seed set.

Suitable stems were harvested and carefully put into large polythene blanket bags in order to minimize seed loss. Mature fruits were then collected. Samples of fewer than about 300 were counted manually, while larger samples were calculated by weighing a counted sample and the whole collection. Flower number was estimated by assessing the number of inflorescences per stem, the number of paracladia per inflorescence and then the number of pedicels per paracladium. The results are shown in Table 4.4. Although, somewhat stochastic I am satisfied that the data produced are valid for comparative purposes. The first point of interest is the huge number of flowers produced per stem, over 190,000 each for the two R. japonica plants from the wild. It will be noticed that these are considerably higher than those produced by the comparable plants P12 and P179 growing in the collection. This difference is I suspect due to competition for space, water and sunlight in plants in the collection. Unfortunately only one male-fertile and one male-sterile clone of R. sachalinensis were included, so it was not possible to make useful comparisons between the relative resource allocations to flowering and fruit production between the sexes. The seed-set of some 2,780

Taxon	Sex	Mean ochrea per infl.	Mean pedicels per ochrea	Mean flowers per infl.	infls per stem	Flowers per stem	Seed per stem	χ seed set	Cult. or wild
<u>R. japonica</u> var. <u>compacta</u> P2a	male-sterile	57	2.58	147.1	42	6176	516	8.35	C
<u>R. sachalinensis</u> P55	male-fertile	139.5	2.65	369.7	153	56,560	2,780	4.92	C
<u>R. sachalinensis</u> P115	male-sterile	205.5	2.33	478.8	209	100,072	11,323	11.31	C
<u>R. japonica</u> P12	male-sterile	182	2.67	485.9	234	113,709	2,800	2.46	C
<u>R. japonica</u> P179	male-sterile	130	2.96	384.8	247	95,045	6,152	6.47	C
<u>R. japonica</u>	male-sterile	146	3.2	467.2	410	191,552	442	0.23	W
<u>R. japonica</u>	male-sterile	179.5	3.37	595.9	322	191,892	283	0.15	W

TABLE 4.4 FLOWER AND SEED PRODUCTION BY REYNOUTRIA SPECIES

seeds in the male-fertile plant of the latter leaves no doubt as to the truly hermaphrodite nature of these plants. The highest recorded seed-set (11,000) was achieved by the cultivated male-sterile R. sachalinensis. Comparison of octoploid R. japonica grown in the collection and in the City makes an interesting comparison. As expected those in the collection had a very much higher seed-set than those in the wild - 2.46-6.47% compared with 0.15 to 0.23%. But the amount of seed produced by the latter (3-400 per stem) is obviously significant, given that such stands consist of dozens of stems, and ought to be more than adequate in terms of potential recruitment of new plants from seed. The relatively small number of seeds produced by R. japonica var. compacta is clearly a reflection of the smaller stature and the less branched more open nature of the inflorescences. In terms of percentage seed-set the ceiling is reached at around 11%, such plants appearing to be covered with seed. Male-fertile R. sachalinensis produced less than half as much as the male-sterile plant in percentage terms, but in terms of numbers of seeds produced the gap was much larger on account of the male-fertile plant having only half the number of flowers per stem of the male-sterile plant.

4.2.5 SEED GERMINATION

The production of seed is only one measure of the reproductive competence of a plant; the ultimate test is of course germination and survival of the seedlings. I have accordingly attempted to germinate seed from wild collected sites in this country and from the Leicester collection. Previous research on Polygonum seed germination was carried out by Justice (1941) with 21 species, 6 of which are the concern of this study. He discovered that all taxa required a period of after-ripening following harvesting in order to break the seed dormancy. In taxa such as F. convolvulus and R. japonica this could be accomplished dry at room temperature, whereas species such as F. cilinodis required 6 months or more in moist conditions.

The germination data are split into two sections, one using whole untreated seeds, the other following the recommendations of Justice. This involved immersing the seed for 5 min in concentrated H_2SO_4 , followed by rinsing in distilled water, neutralization in calcium hypochlorite, and further rinsing in tap water. In this way the pericarp was usually removed, and any remains could be removed by gentle rubbing. The nutlets were then put on moist filter paper at $2^\circ C$ for 17 days in the dark, before being transferred to continuous light at $15^\circ C$. None of the batches of seed had been harvested less than 2 months before the germination tests were conducted.

Table 4.5, though regrettably low on wild-collected seed,

Species	Sex	Wild or cultivated	No. seeds	% germination
<u>R. japonica</u> var. <u>comp.</u> P2B	male-sterile	C	22	91
<u>R. sachalinensis</u> P57	male-sterile	C	36	72
<u>R. sachalinensis</u> P127	male-sterile	W	30	56
<u>R. sachalinensis</u> P61	male-sterile	C	22	41
<u>R. sachalinensis</u> P116	male-sterile	C	18	22
Average male-sterile				48
<u>R. sachalinensis</u> P55	male-fertile	C	9	44
<u>R. sachalinensis</u> P63	male-fertile	C	30	93
<u>R. sachalinensis</u> P62	male-fertile	C	17	70.6
Average male-fertile				69.2
<u>R. japonica</u> var. <u>jap.</u> P25	male-sterile	W	25	76
<u>R. japonica</u> var. <u>jap.</u> P179	male-sterile	C	10	50
<u>R. japonica</u> var. <u>jap.</u> P12	male-sterile	C	35	71
Average male-sterile				66

TABLE 4.5 REYNOUITRIA GERMINATION USING THE TECHNIQUE OF JUSTICE (1941)

amply illustrates the viability of seed produced by both male-fertile and male-sterile plants. In order to assess the role of seed-set in plants outside the collection at Leicester it is necessary to examine germination data from plants growing in the countryside; these are shown in Table 4.6. Of the 16 plants listed, only in the case of P5 was there a known source of Reynoutria pollen in the vicinity, in this case a R. sachalinensis plant.

The variation shown in the germination rates may be affected by the length of time that had elapsed between harvest and the germination test, since the samples used did vary considerably in age, older collections actually tending to give a higher germination rate. Since seed was collected from places as far afield as Scotland, Wales, Hampshire and London, as well as Leicester, it is clear that production of viable seed on British Reynoutria plants is by no means an isolated phenomenon. This is in contrast with Conolly's (1977) findings that seed production was in fact a very rare event. It may however, be possible to reconcile these apparently conflicting findings as will be suggested in the following chapter. Overall a very good germination rate was achieved, but it must be borne in mind that in some of these localities very small numbers of fruits are produced and these represent an infinitesimally small percentage of the flowers produced. In addition to the results in Table 4.6 I have successfully germinated by means of embryo culture seeds from 4 R. sachalinensis colonies in Wales.

	No. Seeds	% Germination
<u>R. japonica</u> var. <u>compacta</u> P99	10	70
<u>R. sachalinensis</u> P127	30	56
<u>R. japonica</u> P25	45	46
<u>R. japonica</u> P8	17	100
<u>R. japonica</u> Barmouth	7	100
<u>R. japonica</u> P18	13	76
<u>R. japonica</u> Llangynadle	2	0
<u>R. japonica</u> P9	20	55
<u>R. japonica</u> P19	14	64
<u>R. japonica</u> P35	23	35
<u>R. japonica</u> Ironbridge	11	45
<u>R. japonica</u> Pontrefelin	14	36
<u>R. japonica</u> P5	43	88
<u>R. japonica</u> P26	17	94
<u>R. japonica</u> P27	2	100
<u>R. japonica</u> West Bridge	21	71

TABLE 4.6 GERMINATION OF WILD COLLECTED REYNOUTRIA SEEDS.

Germination in the Wild

In May 1985 I observed a number of Reynoutria seedlings growing under the R. japonica P12 in the fish-tanks. Not wishing them to grow amongst the other plants I weeded them out. The same year I noticed three more seedlings, looking every bit the aggressive pioneer, growing in the gutter and in between the paving slabs. I could not find them the following year so I do not know whether they fell victim to the weather or to the ministrations of the gardening staff. There is, however, one recorded establishment in the wild of a Reynoutria seedling; this will be fully dealt with in the next chapter.

4.3 DISCUSSION

4.3.1 SEX DETERMINATION IN HIGHER PLANTS

In stark contrast with the animal kingdom the separation of the sexes in higher plants is the exception rather than the rule, less than 10% of plants having separate sexes (Lloyd 1982). The occurrence within this 10% of morphologically distinct sex chromosomes is also extremely rare, though one need look no further than the Polygonaceae for examples of mechanisms involved in sex determination. The diploid species of the subgenus Acetosella of genus Rumex have an XX/XY sex determining mechanism similar to that of Melandrium, with morphologically distinct sex chromosomes. In subgenus Acetosa there are taxa with an XX/XY₁Y₂ system and which produce a sex trivalent in male meiosis. It has been found (Löve 1957) that in this group the Y chromosome has no male determining activity, the sex being determined by the X/autosome ratio. In this case the presence of the Y acts by excluding an X and thus changing the X/autosome ratio in the male direction. In the subgenus Acetosella, on the other hand, the Y chromosome carries a strong male determinant, much stronger than that of Melandrium since it is capable of suppressing the female gene of up to eight autosome complements and seven X chromosomes (Löve 1957).

These two different mechanisms have had an affect on the subsequent chromosome evolution of these two subgenera due to the different effects that polyploidy has on their functioning. Evolution of dioecious polyploids is

effectively ruled out in subgenus Acetosa since autosome/X ratios intermediate between 0.5 and 1.0 produce intersexes. With the strongly male determining Y chromosomes of subgenus Acetosella, dioecious polyploids can, and are, produced up to the octoploid level.

However, it appears that there is a certain in-built lability in sex expression even in those taxa where sex is determined by special chromosomes. Smith (1963), for instance, found that R. hastatulus males ($2A+XY_1Y_2$) contained up to 5% of plants which shared some degree of pistil development, and that some of these could in fact function as hermaphrodites. Other well-known factors affecting sex expression are detailed by Freeman et al. (1980) and indicate cold weather, dry soil, light intensity, high nitrogen, 'rich soil', changes in photoperiod, and 'trauma'. They suggest that this is more significant than the intellectual curiosity that most botanists have treated it as, and that in certain environmental conditions this lability may be of selective advantage to the plant. They postulate that, in a dioecious species growing in an environment consisting of wet and dry patches, where fruit bearing poses a greater stress on female plants, there is a selective advantage for female plants in dry patches to become male and the converse in wet patches.

Sugiura (1936) reported that presence of sex chromosomes of the XY type in Reynoutria japonica on the basis of meiotic disjunction behaviour. Nowadays this sort of evidence is given little credence and there have been no more reports of

sex chromosomes in Reynoutria nor have I been able to find any. In the absence of morphologically recognisable sex chromosomes, sex expression is generally governed by one pair or block of genes and this is, I suspect, the situation in Reynoutria. The gynodioecious condition occurs when a male sterility mutant occurs and becomes established in a population of hermaphrodites. Male sterility can be caused by a single dominant or recessive gene and operate by interfering with pollen formation and release. Various stages of male meiosis may be affected or cells might not reach meiosis, as when the tapetum may persist denying the microspores nutrition; or the pollen may develop but the anthers fail to dehisce.

4.3.2 SEX EXPRESSION IN REYNOUTRIA

The clones of Reynoutria that are naturalized in this country are not, as is often stated, functionally dioecious since they consist of male-sterile and hermaphrodite individuals. The hermaphrodite nature of the latter is not readily discernable when the plants are growing in the wild because the plants are self incompatible (Plate 4.A,B) and consequently usually only minute quantities of seed are produced. The same plants growing at Leicester in the presence of compatible pollen are capable of producing large amounts of fruit. The occasional seed produced in the wild by such plants probably represents the rare breakdown of self-incompatibility. Certainly seed from the hermaphrodite R. sachalinensis at Amroth (P67, P68) has given rise to R. sachalinensis plants with 44 chromosomes. Taxa comprised of male-sterile and hermaphrodite individuals are termed gynodioecious.

I have been able to examine living material from only a small number of R. japonica var. compacta plants and have found them to comprise mainly male-sterile and female-sterile individuals. In spite of the generally immature state of ovaries in the latter, they can occasionally produce small numbers of fruit (Plate 4.1). In the light of this I prefer to call them sub-dioecious rather than gynodioecious.

repeated

The situation with R. japonica var. japonica in this country is a little more difficult, for I know only of male-sterile

plants. Clones morphologically very similar to these male-sterile plants are known from seed grown from Japan (P114) ($2n=44$) and a plant from New York (P169), which I know only as flowers from dried specimens. The Japanese plant is morphologically a hermaphrodite, though due to its late flowering it is not possible to test its female fertility. To a certain extent such observations of foreign material are irrelevant since it is the breeding system of plants growing in this country which is my primary concern, and perhaps a new term should be coined for species which are exclusively male-sterile.

It is in any case difficult to make hard and fast distinctions between dioecy, gynodioecy and trioecy as Kay and Stevens (1986) stated, since in most populations of dioecious plants that have been studied in any detail hermaphrodite individuals have been found. Kay and Stevens do, nevertheless make such a distinction between dioecy, sub-dioecy and gynodioecy based on the relative frequency of hermaphrodites; from 'rare' (dioecious) to 'abundant' (gynodioecious).

Reynoutria

A major consideration with respect to Reynoutria in the British Isles is that as introduced plants they do not constitute a natural population. They were probably introduced from material with a very limited genetic base, with a range of sex-expression that does not necessarily represent the situation in the wild in Japan. This is particularly so with the octoploid male-sterile R. japonica which I suspect all originates from Siebold's nursery at

Leiden. Be that as it may, the peculiar sex ratio of these plants has had some most interesting consequences as will be revealed in Chapter 5. An examination of authentic wild herbarium material at E, K and BM revealed the presence of occasional hermaphrodite individuals from both China and Japan. A male-sterile: male-fertile ratio of 3:1 was found in Chinese material, whereas the sexes were more evenly represented in Japanese specimens. The latter is brought about largely by the Japanese practice of mounting male-fertile and male-sterile branches on the same sheet, which must to some extent reflect the ready availability of both sexes in the wild. This is particularly the case when material is collected from pioneer habitats on volcanic gravel where, as Maruta (1983) has shown, there is considerable recruitment of new plants from seed and hence where one would expect to find an equitable balance of the sexes.

With reference to the apparent excess of male-fertiles over male-steriles shown in Figure 4.1, some further evidence is supplied by a controlled pollination which produced 6x offspring, 6 of which were male-fertile and 1 male-sterile.

4.3.3 THEORETICAL CONSIDERATIONS

Darwin (1884) was well aware of the possibilities of the evolution of dioecy from the hermaphrodite condition, and his contribution is routinely referred to in current papers in this area. His statement 'There is much difficulty in understanding why hermaphrodite plants should ever have been rendered dioecious', is still relevant in spite of the computer simulations and theoretical papers on this subject that now abound.

Theorists such as Charlesworth and Charlesworth (1978) and Lloyd (1982) view the gynodioecious state as being the first step in the evolution of dioecy from hermaphroditism. In order to convert an hermaphrodite to dioecy two separate mutations are required. First a male sterility mutant arises and spreads through the population, at some stage an independant female sterility mutant occurs and spreads through the remaining hermaphrodite plants.

It is of course theoretically possible for dioecy to evolve via a female sterility mutant giving the condition known as androdioecy (hermaphrodites and female-steriles). This as both Darwin and Kaul (1988) noted is extremely rare in nature, Kaul reporting only 10 with the proviso that even these may be a result of year to year variation in sex expression. Two important factors vitiate against it; because of unidirectional pollen transfer androdioecy unlike gynodioecy cannot be maintained by out-breeding advantage and cytoplasmically determined female sterile mutants cannot

be maintained in an androdioecious system.

Charlesworth and Charlesworth (1978) with their computer modelling, concluded that in order for a male-sterile mutant to become established in the first place there must be high levels of self-fertilization and in-breeding depression in the original hermaphrodites. In addition to this the male-sterile plants must show a significant increase in ovule production as compensation for the expenditure saved from the cessation of pollen production. A female sterility gene would then be able to spread through this gynodioecious population if it conferred a moderate increase in pollen output. Its ultimate fate, though, would depend on its mode of action and linkage with the male sterility gene. It would of course be a liability if the two mutant genes could operate independently and additively to produce entirely sterile plants. Lloyd (1982) pointed out, however, that since most male sterility genes act rather late in anther development (usually at meiosis or later) the savings accomplished by male sterility would not be that considerable unless accompanied by a simultaneous reduction in the number of flowers.

02:10.

Lloyd stated that increased heterosis rather than lack of in-breeding depression drives the male sterility gene, though I am not clear whether he is invoking a separate mechanism or the two are analogous with describing the same vessel as half empty or half full.

02:20.

He also stated that there is evidence to suggest that

separate sexes have usually evolved in populations lacking self-incompatibility, since self-compatibility has been found in the 'ambisexual' plants of most gynodioecious species tested. He observed that in gynodioecious taxa the females are usually strictly unisexual whilst the males vary in their seed production; there is, he suspected, little to gain from being nearly male-sterile since even small quantities of pollen would lead to a lot of self pollinations.

The advantages of separate sexes for the male-sterile plant are supposed to be increased out-crossing and hence heterosis of the seed, plus an increase in seed production due to the redirection of reproductive effort formerly required for pollen production. There is coupled with this the somewhat dubious advantage in bird dispersed seed plants that a better display of seed will lead to better seed dispersal. The advantages to male-fertile plants are less obvious, but must include increased contribution to seed production without the responsibilities of having to bear it, although any increase in cross-pollination would need to be balanced against the loss of seed formerly produced by self-fertilization. There are two quite important disadvantages in having separate sexes. In scattered populations or in instances of long range dispersal there is a good chance of no seed set. Secondly, the regular pollinators must be taken into account, since if their only interest is in collecting pollen, male-sterile flowers would be rendered unattractive.

Less than 10% of higher plants exhibit the phenomenon of separate sexes, and the infrequent occurrence and scattered taxonomic distribution not only suggest that gender dimorphism is a derived condition, but also that it is perhaps only in certain specific circumstances that it confers any real benefits. Gynodioecy on the other hand could be viewed as superior to dioecy or monoecy since it contains the best elements of both. Indeed Stevens (1988) referred to the notion of stable and unstable gynodioecy, and it is only the latter that readily evolves into full dioecy.

How, then, does this relate to Reynoutria and Fallopia? It is difficult to imagine the highly derived cleistogamous condition of some accessions of F. convolvulus to be heading to dioecy. F. baldschuanica is strongly self-incompatible and as such would not gain much in the way of increased heterosis from the dioecious condition. The other diploids are all self-compatible hermaphrodites. R. sachalinensis is the most convincing example of a gynodioecious taxon that I have, and in the small sample studied the male-sterile plants produce considerably more seed than do the hermaphrodite individuals. If the theories are correct one would expect a higher seed-set in the male-sterile for the reasons stated previously. This is not universally the case, since Stevens (1988) found that in the gynodioecious Saxifraga granulata L. male-sterile plants produced significantly less seed than hermaphrodites, and he cited two further examples of this phenomenon. He also gave information about the relative properties of hermaphrodite

and male-sterile plants in a population of S. granulata: 23% male-sterile, 63% hermaphrodite and 4% which were termed intermediate. It is not, of course, possible to make meaningful comparisons between this and the situation in an introduced species such as the Reynoutrias.

Kaul (1988) listed several hundred gynodioecious taxa and detailed what is known of the genetic control of male sterility in these plants. This, it seems, can vary greatly even within the same genus, suggesting that it has occurred independently on a number of occasions during the course of evolution. Control may be purely genic with one, two or even five separate genes involved, or a result of an interaction between nuclear and cytoplasmic male sterility determinants. Since self-ⁱⁿcompatibility and male sterility are independent genetic systems that function to promote out-breeding, the two are rarely found together. Reynoutria is not alone in this respect since Armeria maritima L. Hirschfeldia incana (L.) Lagreze-Fossat Limonium vulgare (L.) and Plantago lanceolata L. also combine self-incompatibility with gynodioecy. Gynodioecy has also been reported from the related taxa Fagopyrum esculentum Moench, F. tartaricum (L.) Gaertner, Polygonum historia (sic) = P. bistorta L. and P. viviparum L. (Kaul 1988).

The extent to which theories of the evolution of dioecy via gynodioecy have any relevance to the position in Reynoutria is questionable.



Plate 4.1 Seed set on male fertile plants

Good fruits produced by an apparently female sterile *R. japonica*

var. compacta X 3.6

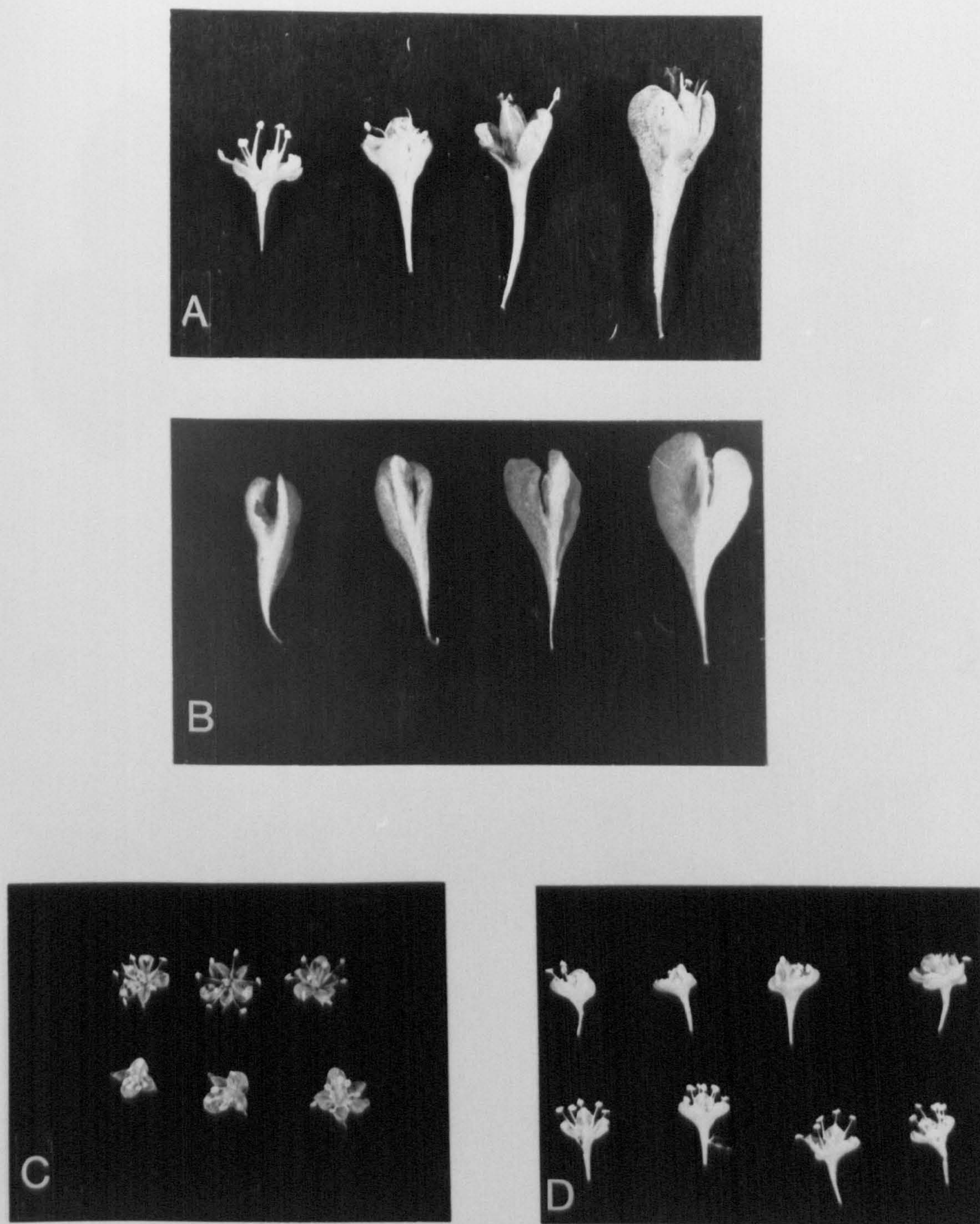


Plate 4.2 Flowers and fruits from male fertile and male sterile plants

A Stages in fruit formation in hermaphrodite *Reynoutria* P51b X 2.9

B Fruits produced by male fertile P79c left, and male sterile fruits P79a ,right. X 3.7

C,D Flowers from the *R.sachalinensis* plant from Osgathorpe to show variation in stamen and gynoecium development X 1.9

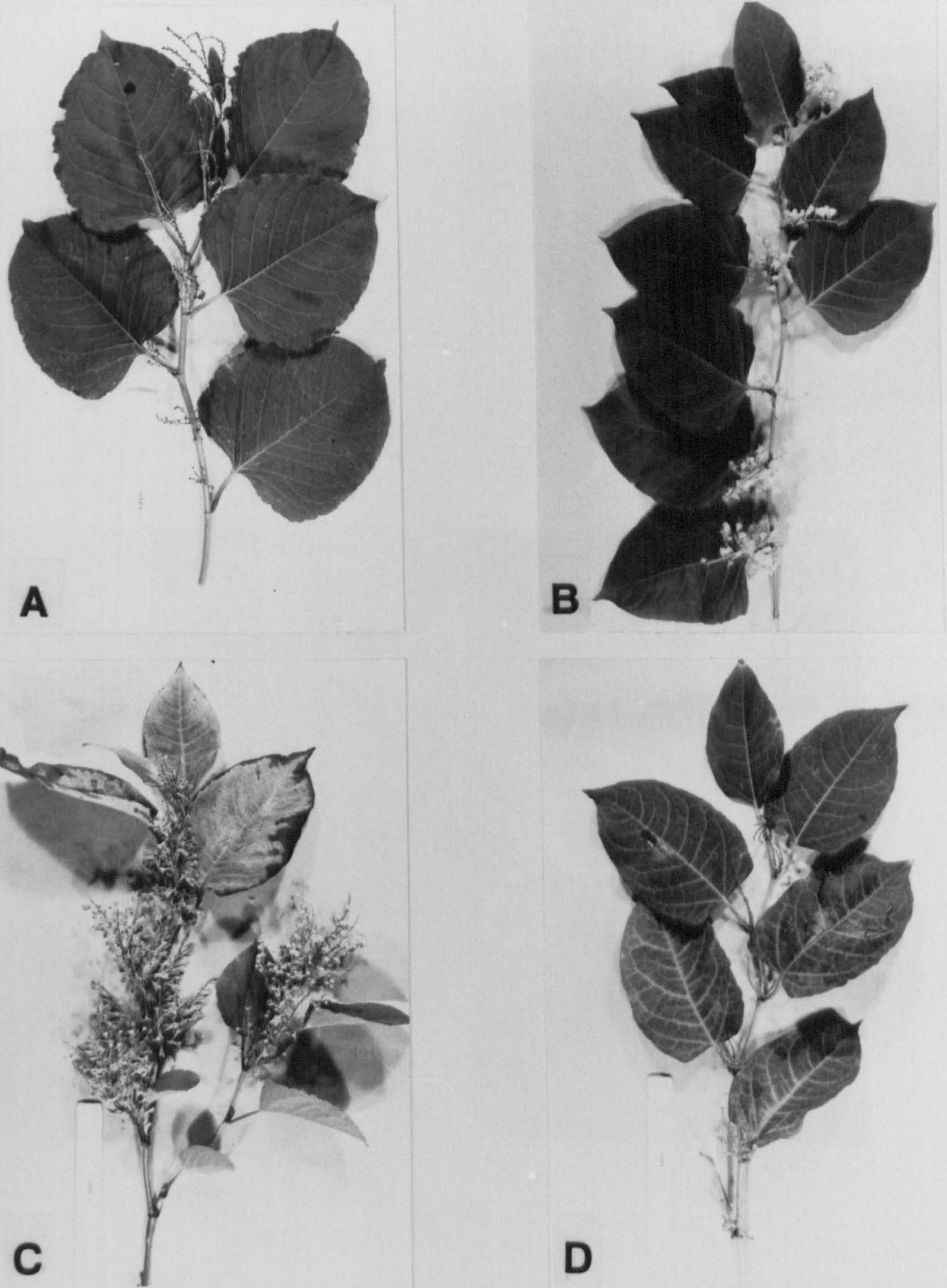


Plate 4.3 Seed set

- A,B** Comparison of seed set between male fertile and male sterile plant of the same genetic constitution. Male fertile P79c **A** with one fruit, and male sterile P79a **B** with good seed set X 0.15
- C,D** Two branches of hermaphrodite *Reynoutria* P51b, **C** a late flowering branch with a high seed set, **D** an early flowering branch with little seed set X 0.17

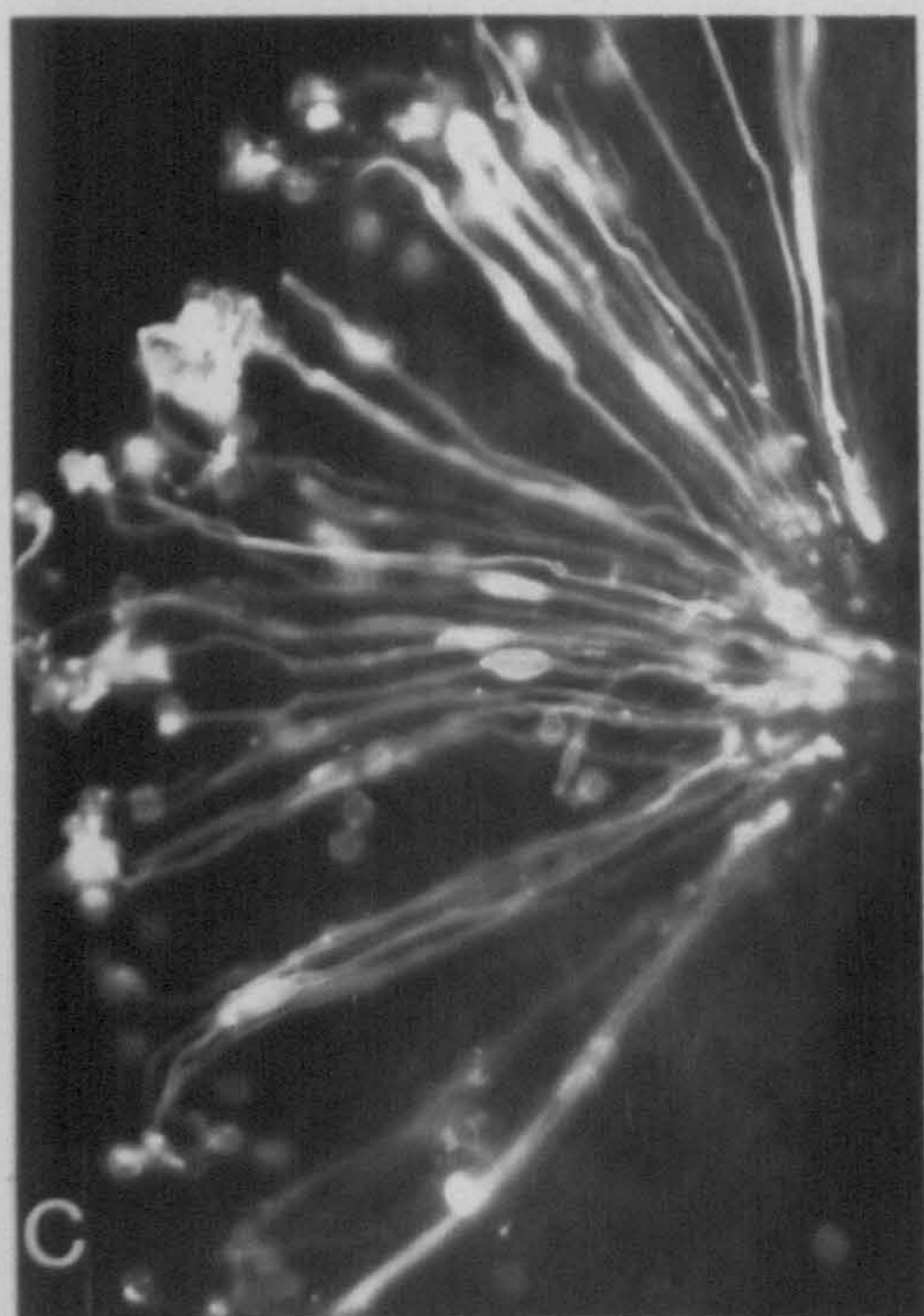
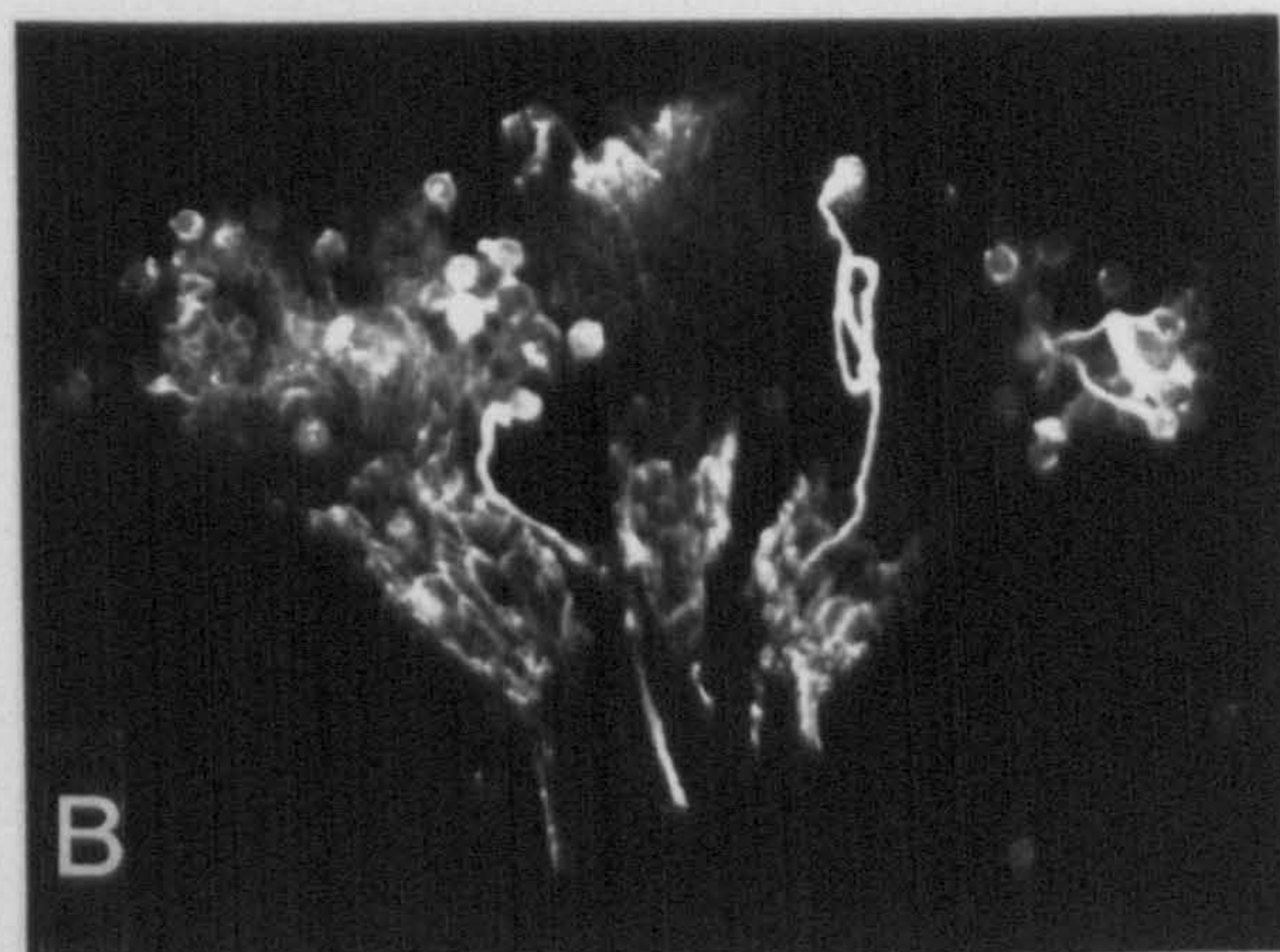
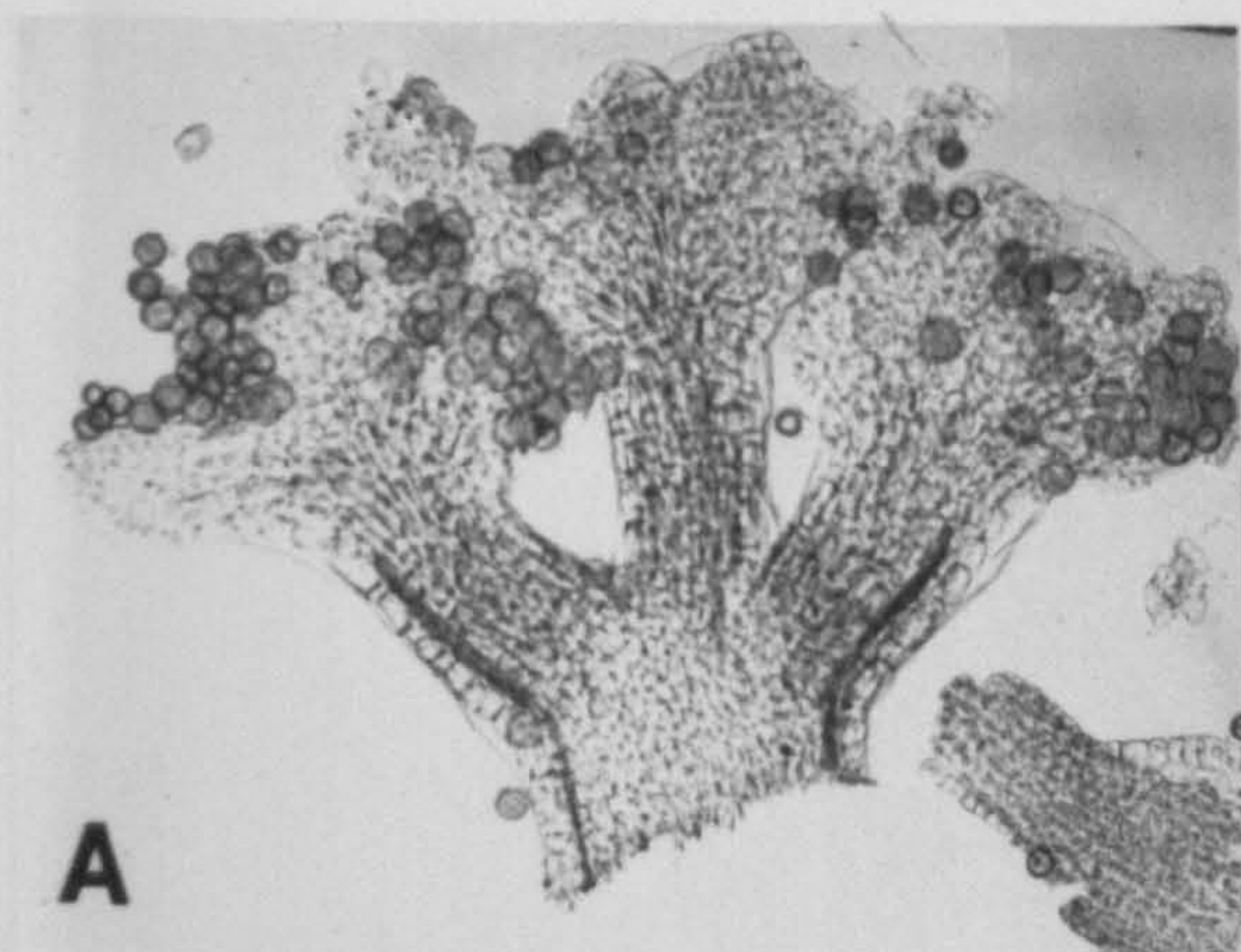


Plate 4.4 Pollen Germination - Incompatible Reactions

A,B The same stigma viewed **A** by bright field and **B** by U.V. Note good pollen adhesion but poor germination and irregular tube growth.
P78a selfed X 75

C,D Side view and polar view of self pollinated *F. baldschuanica* P163 viewed by U.V. Note good germination but irregular tubes with swellings, terminating abruptly at the end of the stigma

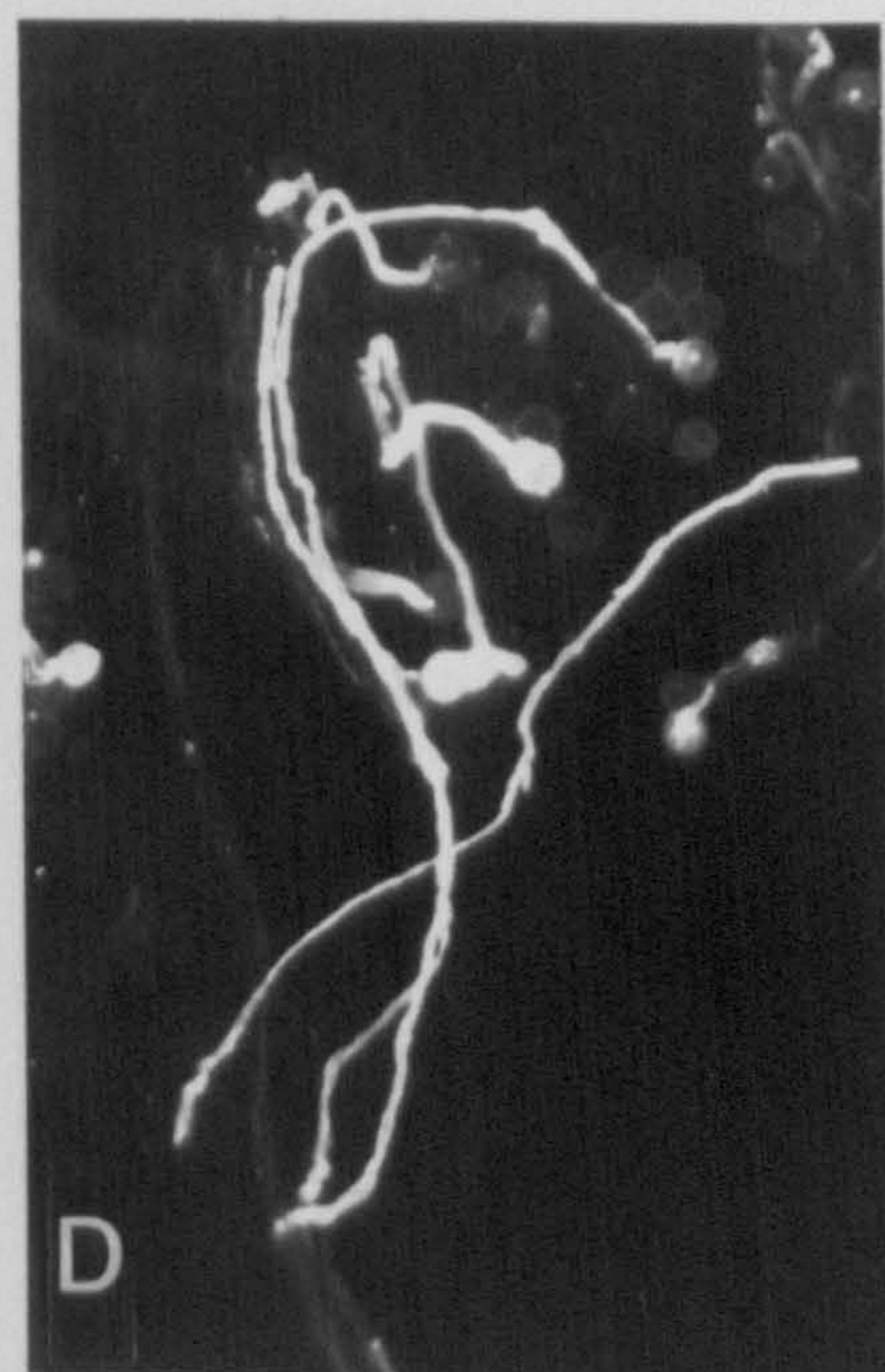
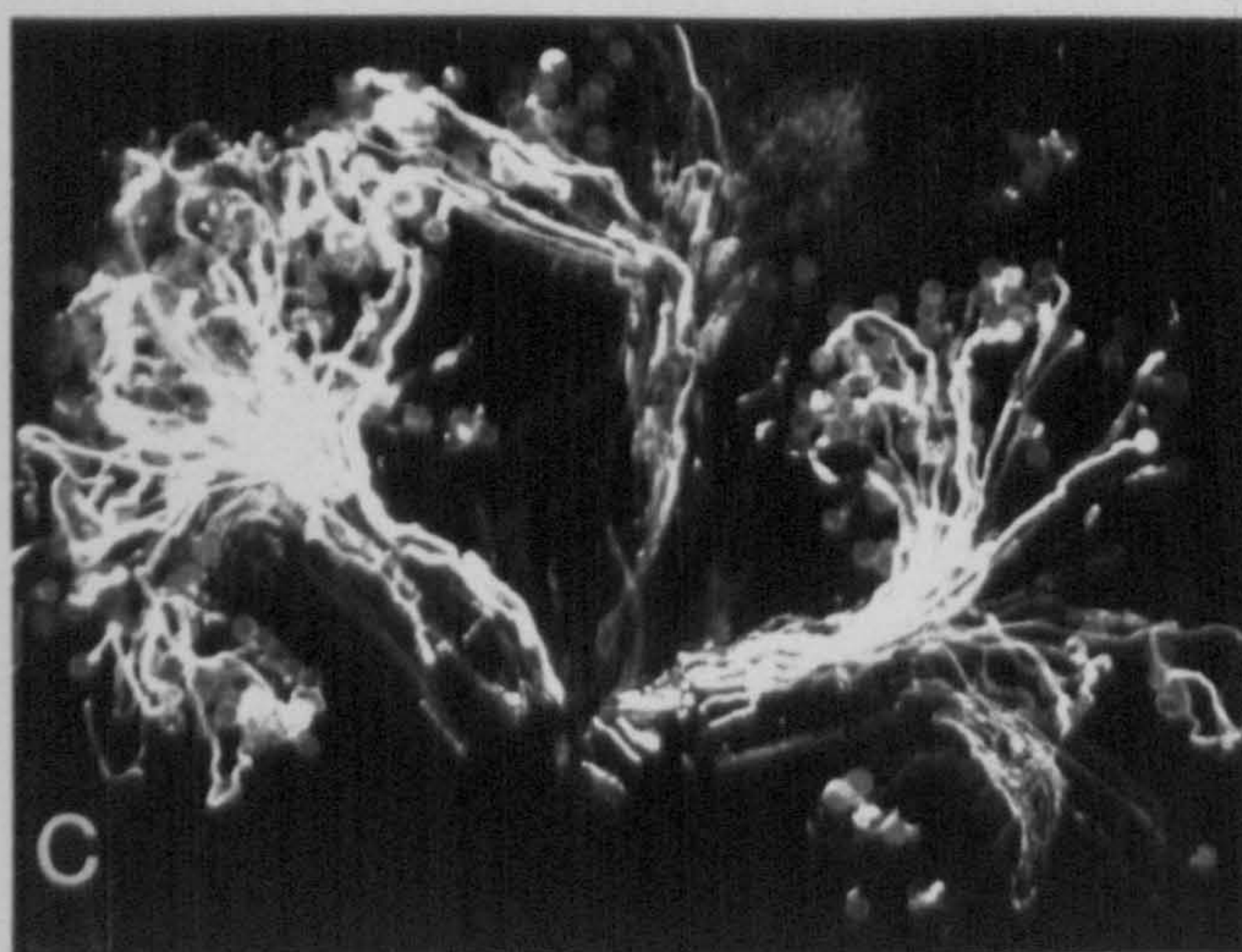
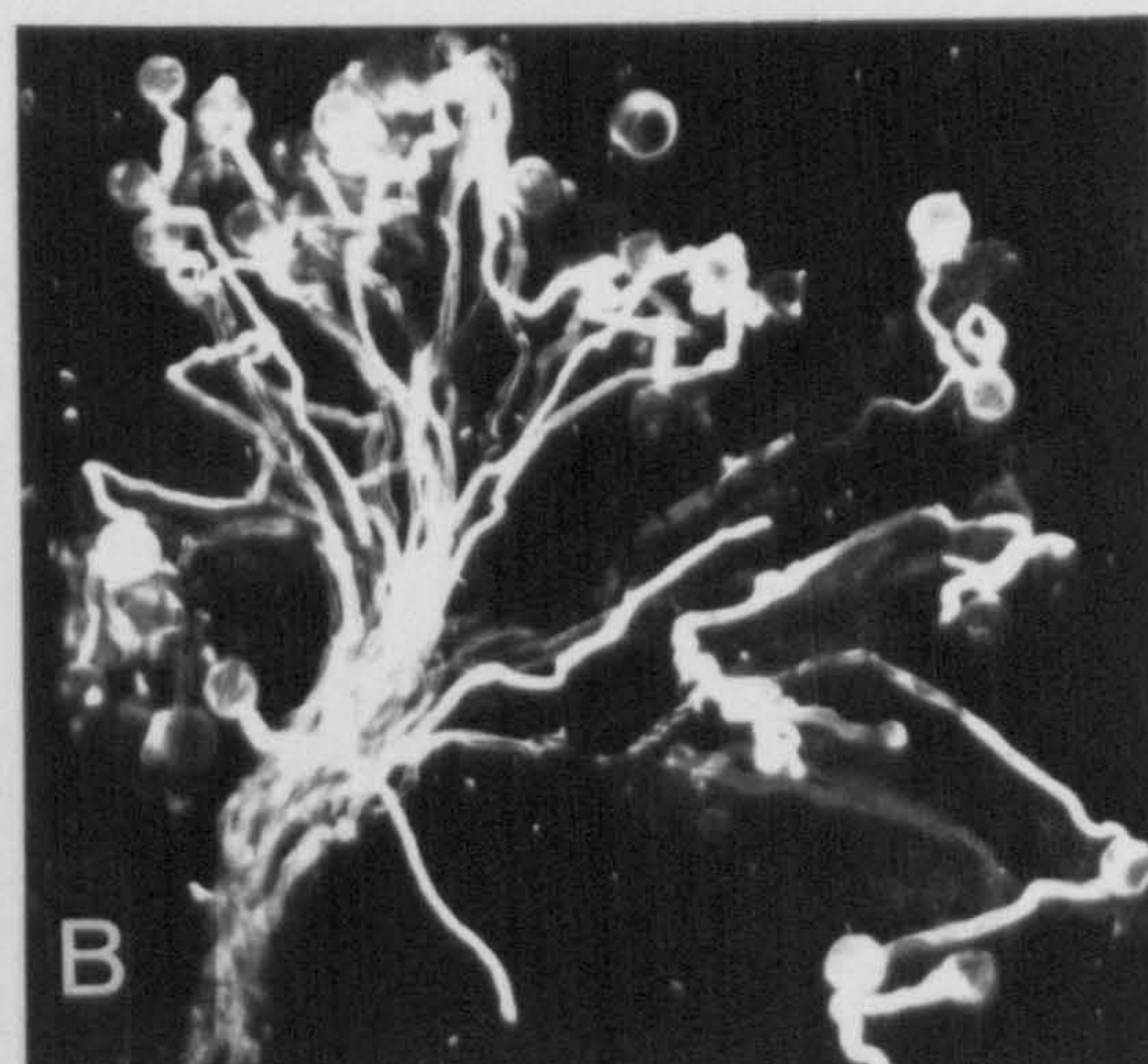
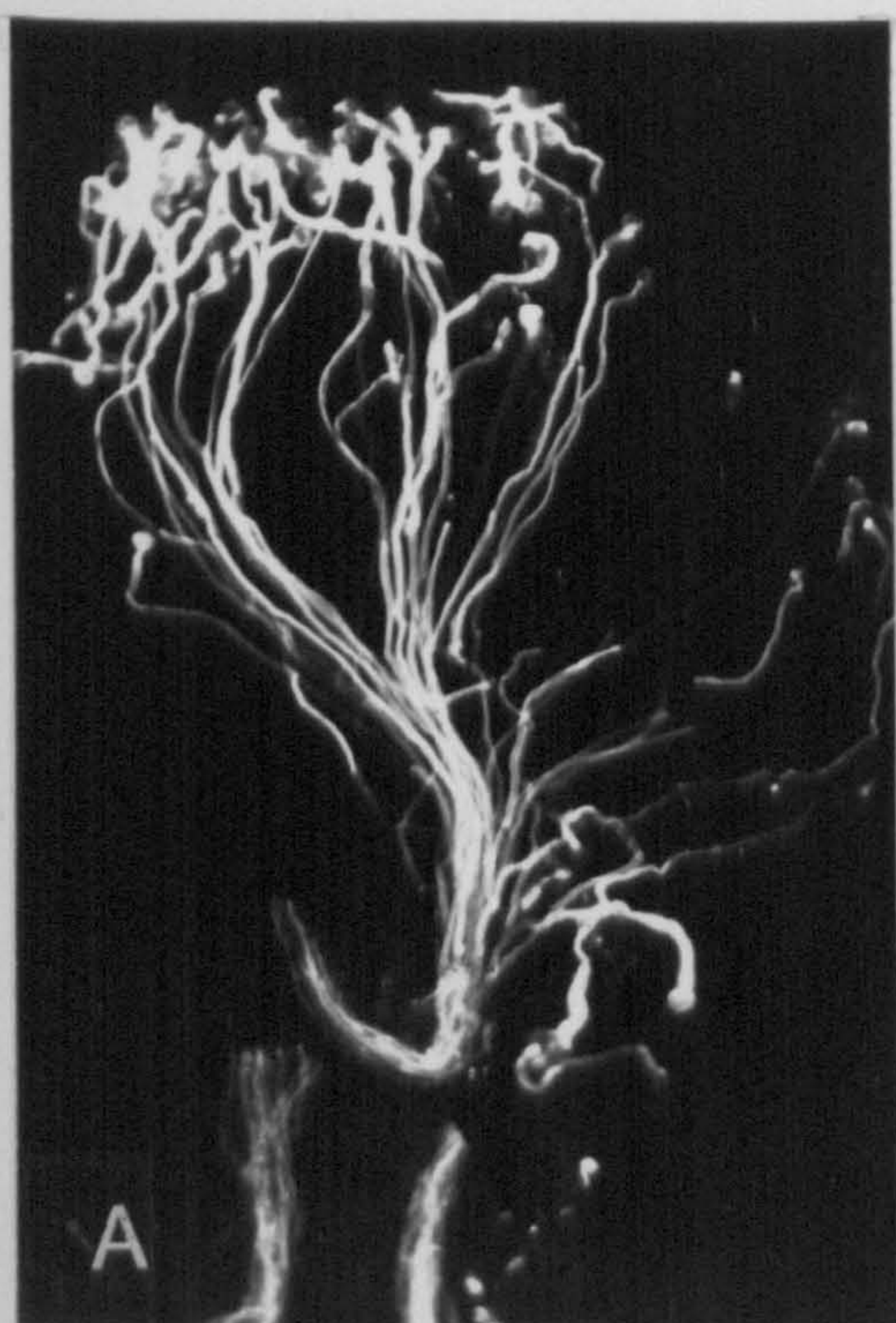


Plate 4.5 Pollen Germination

- A** *R. japonica* P179 stigma showing compatible pollen reaction X 75
- B** *R. sachalinensis* P55 stigma showing good pollen tube growth and germination X 75
- C** *R. sachalinensis* P68 with good germination and growth X 75
- D** *R. sachalinensis* P68 selfed, low germination with tubes terminating in irregular swellings X120

Chapter 5

Hybrids and hybridization

5.1 INTRODUCTION

In this chapter the results obtained in the preceding chapters concerning the various species are brought together and contrasted with the results presented here for the hybrid taxa. Descriptions of the hybrids are given and their morphology and mitotic and meiotic characteristics discussed. Additional results are presented in the area of breeding behaviour, particularly concerning the in vitro and in vivo germination of pollen. I am presenting the data concerning both species and hybrids in this section, so that the former act as the controls.

The study of Reynoutria hybrids does not have a very long history. Chrtek and Chrtková (1983) described an R. japonica x R. sachalinensis hybrid in Czechoslovakia as R. x Bohemica. Bailey and Conolly (1985) also mentioned the existence of the same hybrid in the British Isles. Schmitz and Strank (1985) mistakenly named an R. sachalinensis plant in Aachen (Germany) as R. x vivax, whilst Bailey (1988) described the discovery of the first Reynoutria x Fallopia hybrid in the wild (P.164 Haringey). Of these it is only Bailey and Conolly that have made a cytological study of the plant. This apart, an examination of published chromosome counts for Reynoutria reveals two counts of circa 66 (Menshikova 1964), and more than 60 (Zhukova 1967)) that are suggestive of hybrid origin, although of course they could be inter-ploidy level R. japonica crosses.

Once the hybrids had been discovered it was necessary to

learn something about their distribution and frequency in the British Isles, so a number of Reynoutria clones from all over the country were examined cytologically and morphologically. Although a plant morphologically intermediate between two species may be suggested as a hybrid the case for hybridity is much stronger if the hybrid can be artificially reconstituted. An examination of the meiotic behaviour of the various hybrids has also been carried out to see if any genome homologies are revealed. The pollen fertility and seed production and fertility has also been examined, since if these plants have spread by seed such information is important.

The inter- and intra-specific Reynoutria hybrids and the Reynoutria x Fallopia hybrids, including some combinations which have not yet been found in the wild, are shown in Figure 5.3.

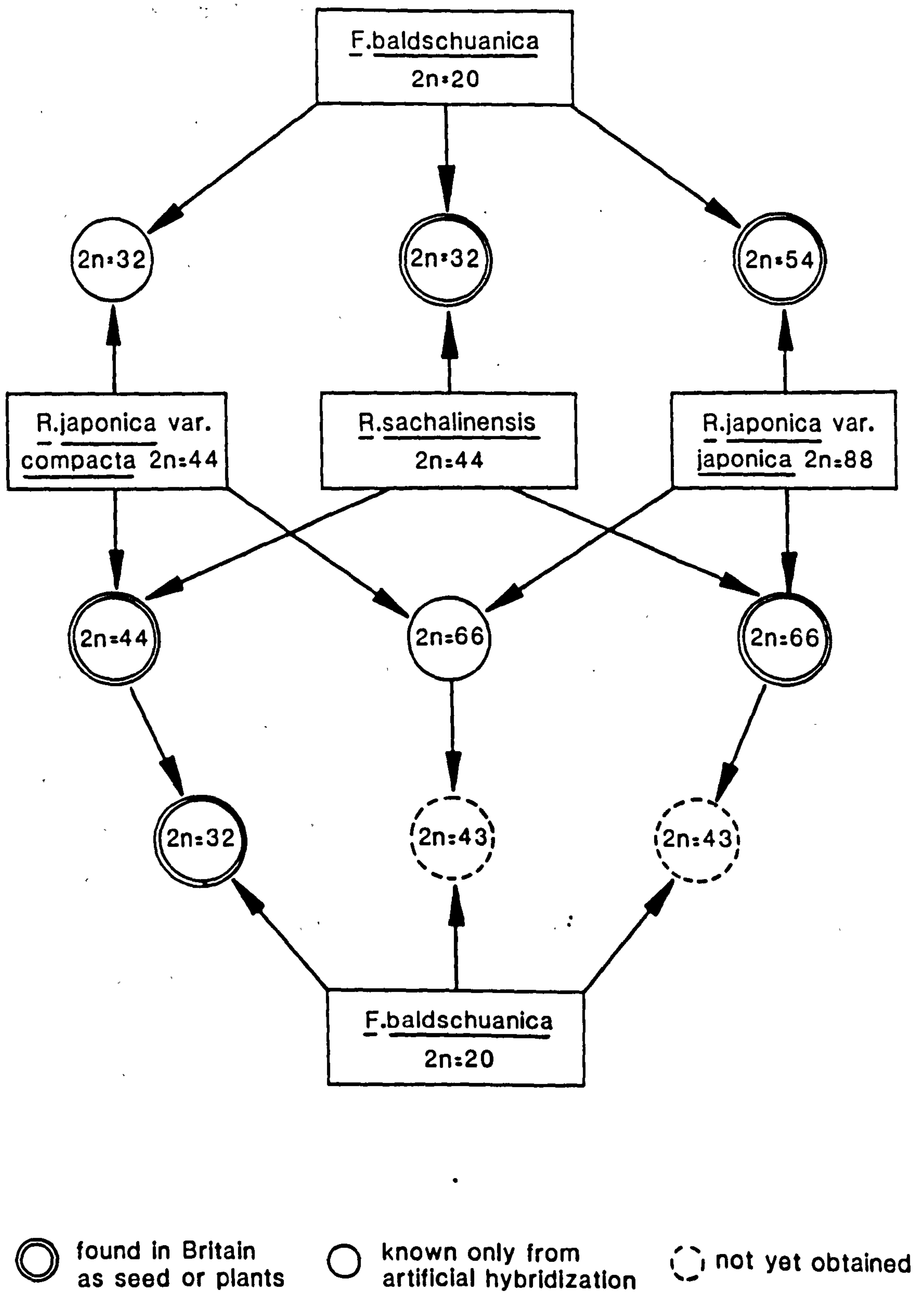


Figure 5.3 Possible *Reynoutria* and *Fallopia* hybrid combinations

5.2 MATERIALS AND METHODS

5.2.1 POLLEN STAINABILITY

Anthers from several flowers per clone were dissected in a drop of Müntzings carmine, and the cover slip sealed with nail varnish. Preparations were left at least 24 hr before examination. Measurements of pollen diameter were made using an eyepiece graticule.

5.2.2 IN VITRO GERMINATION OF POLLEN

Various concentrations of sucrose from 5 to 20% were made up in Gamborg's B5 medium (without hormones) and with 0.01% boric acid. This was dispensed into 2cm watch glasses to which had been added 10 mature Reynoutria stigmas. A small piece of cellophane was floated on the solution and the pollen dropped on top of this. The watch glasses were fixed to 3 x 1in microscope slides with 'Blu tack' and incubated at room temperature in a moisture chamber (Figure 5.4). The whole slide could be examined under the microscope, or the sheet of cellophane could be removed and mounted on a slide. Incubation was usually for 2 to 4 hr.

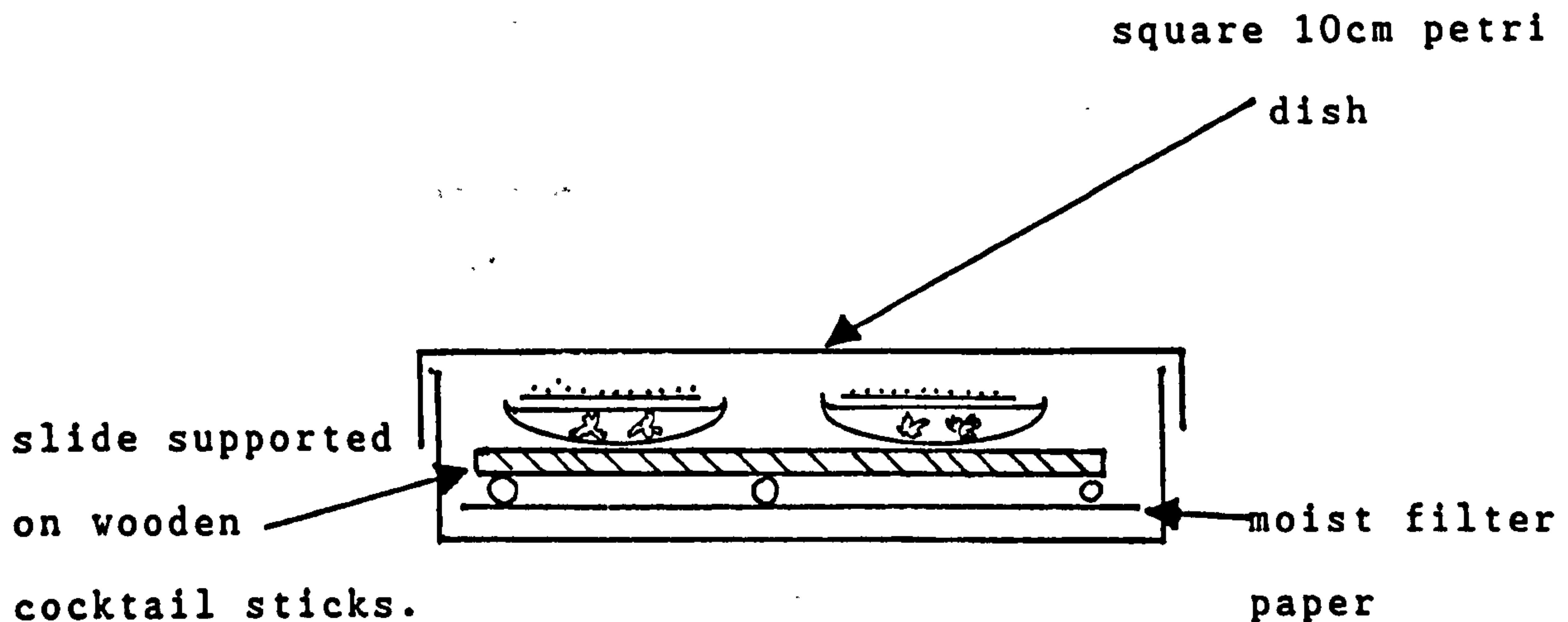


Figure 5.4 POLLEN GERMINATION APPARATUS

5.2.3 OBSERVATIONS OF POLLEN GERMINATION AND PENETRATION

- 1) Selected inflorescences were bagged before the onset of flowering.
- 2) Newly opened flowers were pressed into 0.75% agar containing Gamborg's medium (without hormones) and 2% sucrose. This was stored and dispensed aseptically into 5cm diameter petri dishes.
- 3) Anthers at an appropriate stage of dehiscence were removed under a dissecting microscope and pollen sprinkled on to the test stigmas.
- 4) The agar plates were incubated at room temperature for periods ranging from 4 to 48 hr.
- 5) The pollinated flowers were then fixed and stored in 3:1 glacial ethanol:ethanoic acid.

- 6) Flowers were then treated with 8N NaOH for 30 min at 60°C, and rinsed in distilled water several times.
- 7) Flowers were stained in 0.2% aniline blue made up in 0.3M K_3PO_4 for a minimum of 30 min.
- 8) Stigma and style were cut off and mounted on a slide in a drop of glycerol. Gentle pressure was used after the addition of a cover-slip. (steps 6, 7 and 8 follow the technique of Chu and Harberd 1970).
- 9) Preparations were then examined using a Zeiss standard microscope fitted with fluorescence attachment 2FL, and filter set 487709 (Blue excitation).

When using male-fertile flowers it was not practicable to emasculate them whilst on the plant. This inevitably meant that some self pollen reached the stigma; hence in all such crosses an uncrossed set of flowers from the same batch was used as a control.

5.2.4 EMBRYO CULTURE

Gamborg's B5 medium without kinetin and sucrose (Flow laboratories), with the addition of 2% sucrose and gelled with 1% oxoid Agar No. 3 was used. This was steamed, distributed to the final containers (3 x 1in glass specimen tubes), capped with oxoid aluminum caps, autoclaved and sloped.

Fruits were first imbibed for 4 hours or upwards and then the pericarp was removed and the seed dipped in 70% IMS and placed in 10% bleach (Domestos thin) for ten minutes. After

3 or 4 rinses in sterile distilled water, the embryos were aseptically dissected out using a binocular microscope and watch-maker's forceps. Embryos were pressed lightly into the medium, the caps sealed with Nescofilm, and the tubes incubated at 25°C in the light. When old enough the germinated seedlings were transferred to a sterile mixture of 12 part sand: 1 part Levington's compost and kept at very high humidity for several days; the humidity was then gradually reduced until plants were able to withstand the rigours of our glasshouse.

If, as was sometimes the case, the radicle was damaged during dissection, causing shoot growth without root growth, such seedlings were transferred to medium containing 0.1 mg l^{-1} IAA in addition to the normal medium.

5.2.5 HYBRIDIZATIONS

The various methods that have been used are listed below:

1. Cut branches with most leaves removed and covered with large polythene bags sealed with wads of cotton wool were used successfully as female parents for some of the earlier Reynoutria hybrids.
2. Inflorescences in the field were bagged with glassine bags sealed around the stem with non-absorbent cotton wool and secured with a wire plant-tie before the onset of flowering. Fresh pollen was transferred to receptive stigmas with a clean paint-brush reserved for this purpose. In the case of hermaphrodite taxa the bags

were checked twice a day and the flowers emasculated prior to anthesis.

3. Hybridization attempts using Fallopia convolvulus and F. dumetorum presented special difficulties. It was discovered that in F. convolvulus the anthers dehisced over the stigma several days before the flower opens. It was thus necessary to carry out emasculations of the young tightly closed buds. The strongly keeled 3 outer perianth segments proved impossible to open without damaging the flower, and it was also difficult to remove the 8 stamens without damage to the ovary or rupture of the anthers. In spite of these difficulties about 75 crosses were attempted with F. convolvulus as the female parent. Emasculated inflorescences were marked by a piece of coloured cotton tied loosely directly below the flower; vaseline was smeared on the damaged flowers in most cases. Non-emasculated flowers were removed from time to time.
4. Attempts were made at the in vitro pollination of F. convolvulus and F. dumetorum in a move to avoid difficulties presented by large numbers of emasculations. Tightly closed flower buds were removed, surface sterilized as listed under embryo culture technique, and the ovary removed aseptically and placed on the following medium:

Nitsches medium (Nitsch, J.P. and Nitsch C., 1969)
with the following combinations of growth hormones:

1. 2.3 μ M 2,4 dichlorophenoxyacetic acid

2. 14 μ M Kinetin

3. 2.3 μ M 2,4 dichlorophenoxyacetic acid + 14 μ M Kinetin

4. 2.3 μ M 2,4 dichlorophenoxyacetic acid + 10 μ M Kinetin

5.3 RESULTS

5.3.1 EXTENT OF HYBRIDIZATION

5.3.1₁ Occurrence of wild hybrids

Morphological and cytological investigation of a number of Reynoutria clones in the British Isles has revealed that hybrids between R. japonica and R. sachalinensis are to be found at low frequencies in all parts of the country, Figures 5.5-5.6. Possible and actual hybrid combinations are shown in Figure 5.3.

R. japonica var. japonica x R. sachalinensis 2n=66.

This combination is the most commonly found, (distribution map Figure 5.5) and to date I have examined material from 21 localities in the British Isles. These range from Cornwall in the South, to Galway in the West and Ayrshire in the North. The concentration in Wales is probably due to its being more intensively searched than other areas. The Eastern part of the country has few records and none in Lincolnshire and East Anglia. This may be a result of the less frequent occurrence of Reynoutria species in the East as commented on by Conolly (1977). In any case one cannot place too much significance on the distribution of alien plants, particularly ones that do not usually reproduce by seed, since any differences in climatic preference are likely to be masked by the idiosyncrasies of its initial distribution and its rejection as a garden plant. Some of

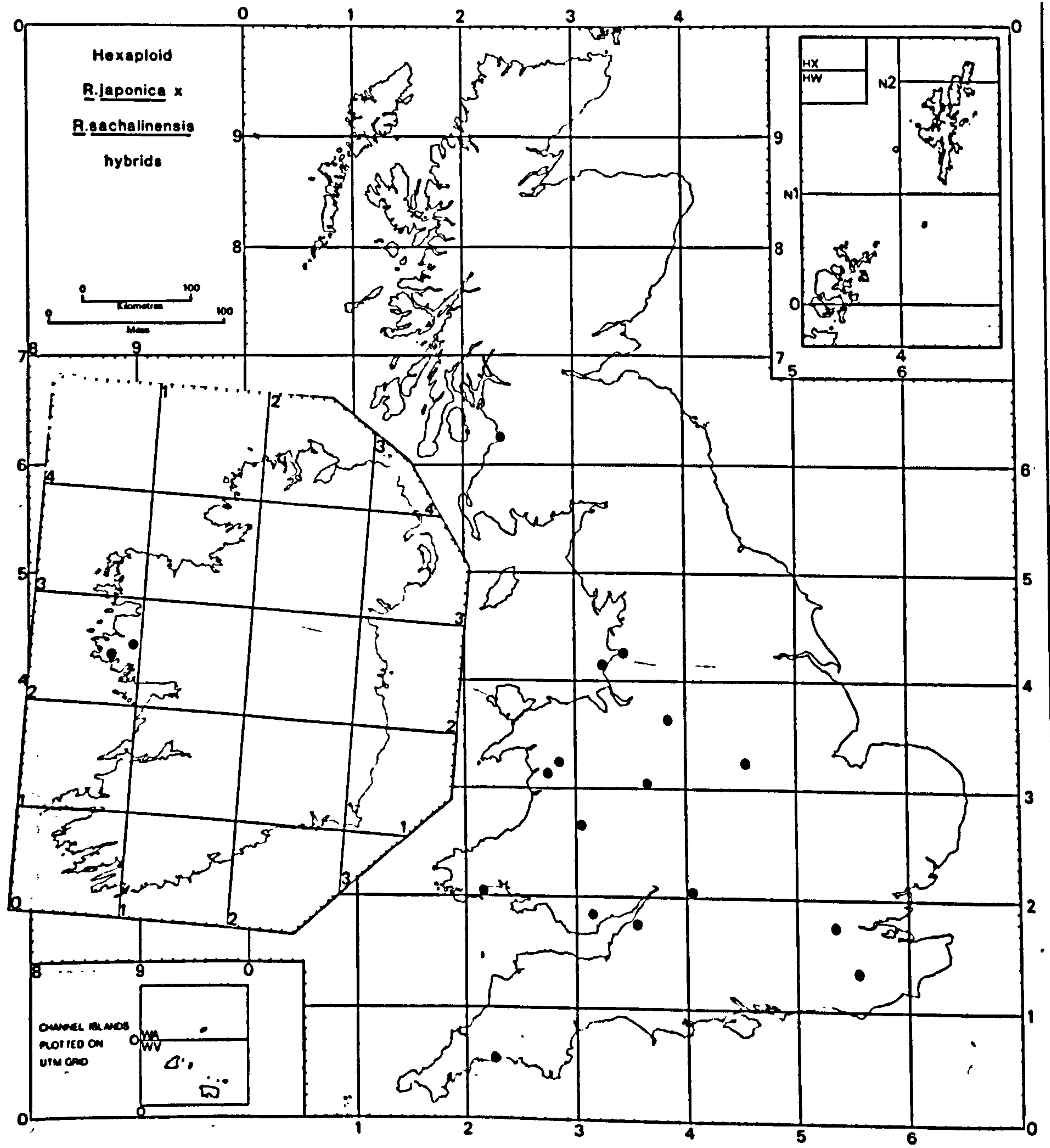


Fig. 5.5 Distribution of 6x *R. japonica* x *R. sachalinensis* hybrids.

Fig 5.5 Distribution of 6x *R. japonica* x *R. sachalinensis* hybrids.

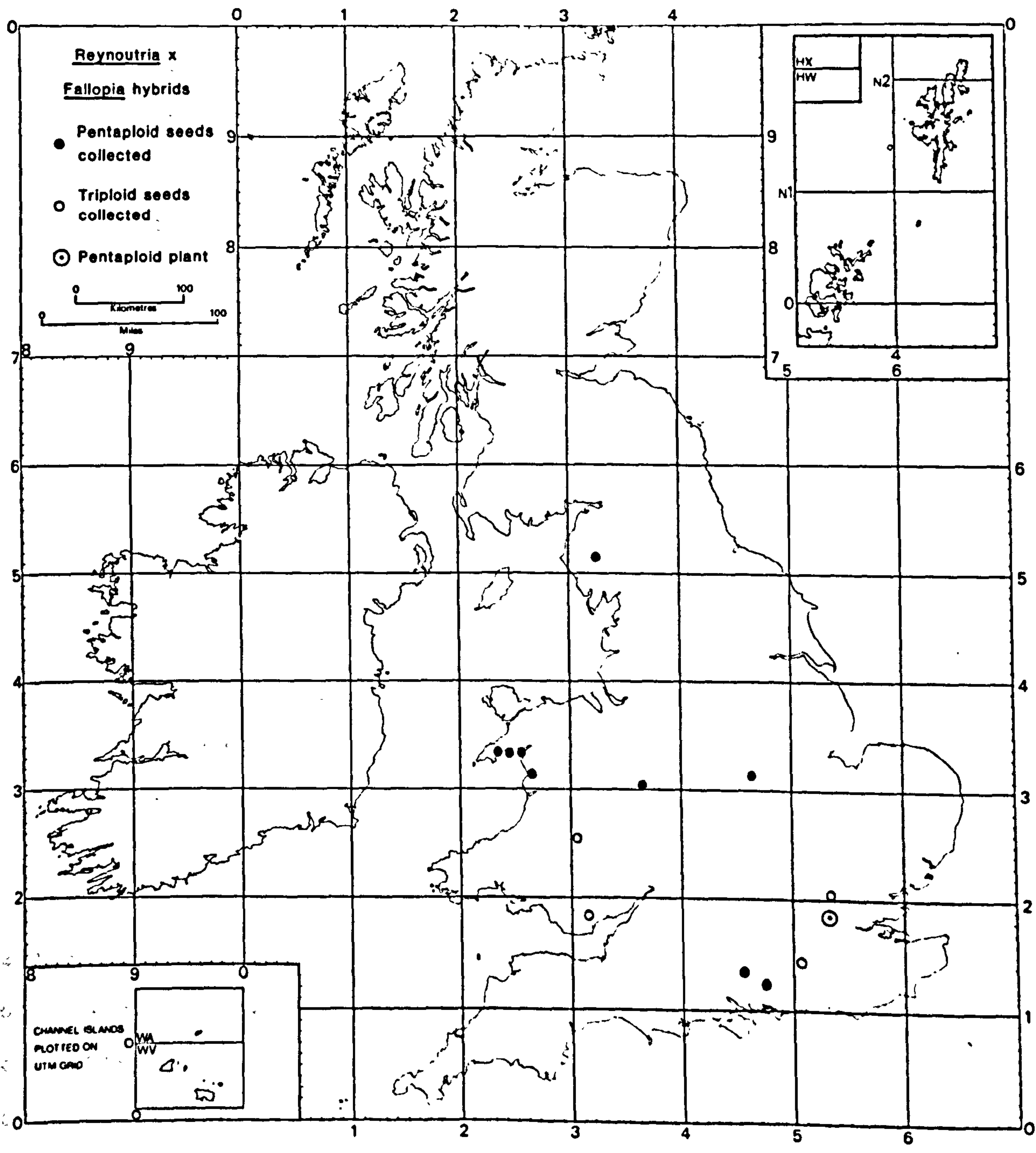


Fig. 5.7 Distribution of 3x & 5x *Reynoutria* x *Fallopia* hybrids.

the coastal populations such as Amroth (P67,8) and Southport (P165) may well have been deliberate since Reynoutria species were advertised by von Siebold (see Frontispiece) as being suitable for stabilizing sand.

Other botanists and I have identified further hybrids between these two species on morphological grounds from herbarium material, but as one cannot distinguish reliably between the hexaploid and tetraploid hybrids on morphological characteristics alone I have not included these on the distribution maps.

Although one does sometimes come across R. sachalinensis and R. japonica growing together it is only at Caerynwch Hall in Wales (P5) that the R. sachalinensis is male-fertile. Hence this is the only locality where I have collected seed from a male-sterile R. japonica and found it to be a hybrid with R. sachalinensis. This is also one locality that I suspect the hybrid has arisen spontaneously - although the assistance of the gardeners cannot be ruled out.

Plate 5.22a,b shows electron micrographs of male-sterile and male-fertile flowers produced by plants P75c and P75d, (products of one set of artificial hybridizations).

Description

Herbaceous perennial, strongly rhizomatous growing to 3-3.5m in suitable locations. Clearly intermediate between its parents but readily distinguishable from them on the following grounds. Leaf shape and size; ovate with cuspidate apex intermediate in size; up to 24.5x17cm

(length, breadth ratio 1.3-1.6) basal leaves with cordate to subcordate bases (unlike the strongly cordate R. sachalinensis leaf bases and the truncate ones of R. japonica). Cuticle and trichome types; strikingly intermediate trichomes are found (Plate 5.19), compare with parental types (Plates 2.3b, 2.6d). An intermediate degree of cuticular striation is also found (Plate 5.19), parental types Plates 2.3c, 2.4b and 2.6b,c.

R. japonica var. compacta x R. sachalinensis 2n=44

This is a much more uncommon combination and has been found at only 5 localities which exhibit (apart from the Durham plant) a more restricted range of distribution (Figure 5.6). I have never come across seed of this combination in the wild, and know of no locality where the two parent plants grow together. Since both R. japonica var. compacta and R. sachalinensis have male-fertile and male-sterile cytodemes I have been able to make reciprocal hybrids for this combination.

Description

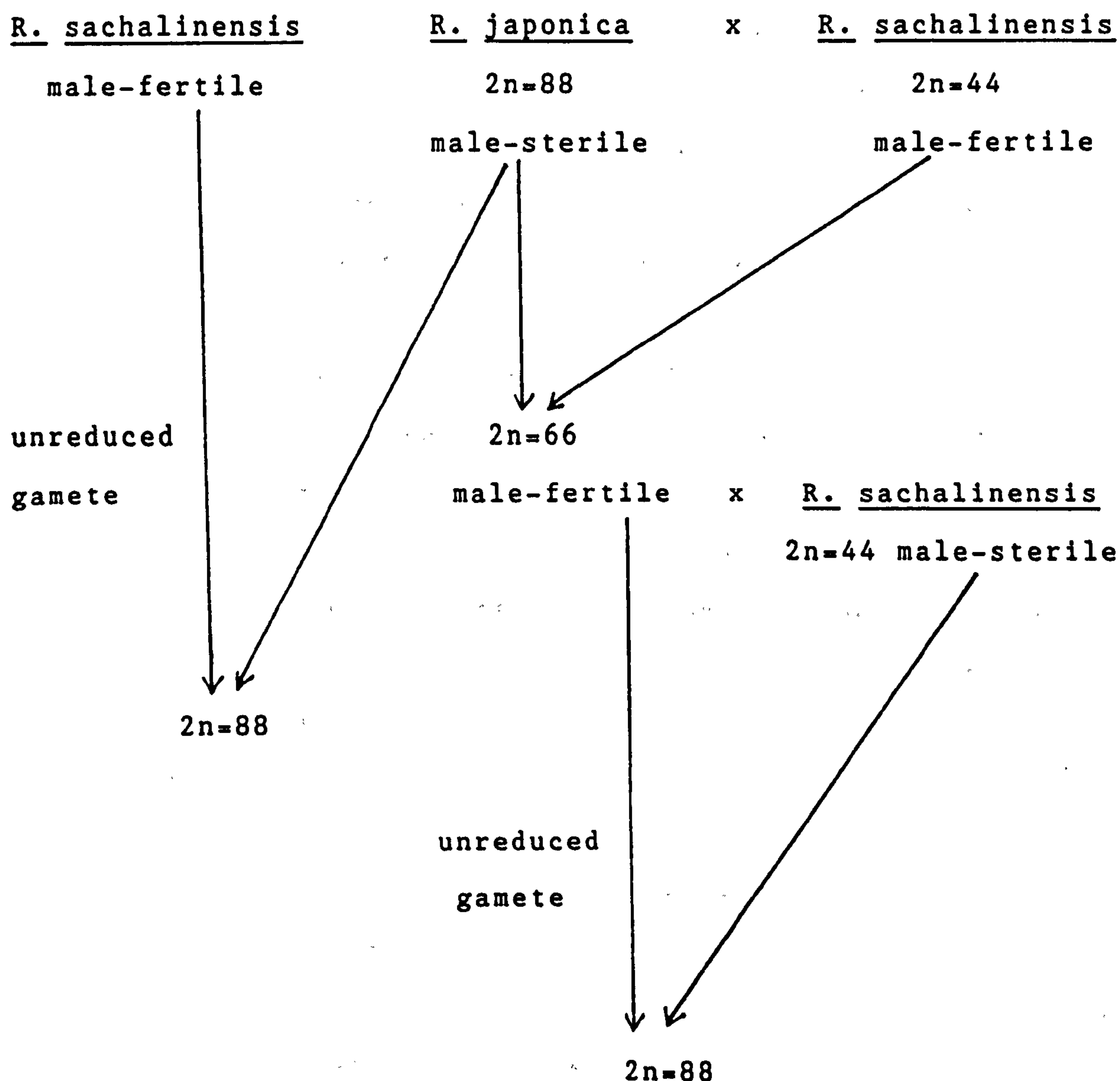
This hybrid is extremely similar to the hexaploid R. japonica var. japonica x R. sachalinensis and I have no absolute means of distinguishing the two in the wild, apart from of course the chromosome number. That aside the artificial hybrids at Leicester show certain differences according to whether R. sachalinensis was the male or female parent. Those with R. sachalinensis as the female parent are readily distinguished by their smaller stature up to 1.5m and by their much earlier onset of flowering; early to

mid-August rather than mid to late September, which characterises not only the tetraploid hybrids with var. compactum as female parent but also the other Reynoutria taxa apart from R. japonica var. compacta.

All the tetraploid hybrids tend to have broadly ovate leaves with cuspidate apex up to 20x20cm (length/breadth ratio 1.0-1.25). Some also have the darker thicker leaves with crimped edges (Plate 4.3a) characteristic of R. japonica var. compacta. That this is of somewhat sporadic occurrence is evidenced by Plate 4.3b which shows another artificial hybrid from the self-same cross, but with much less crimping of the leaf margins.

Octoploid R. japonica x R. sachalinensis 2n=88

This plant is known only from a single location in Wales (Dolgellau) (Figure 5.6) where it grows in company with a hexaploid hybrid of the same combination. It is also unusual in that it is the only male-fertile octoploid known in the British Isles. This permutation is not listed in Figure 5.3 as the inclusion of all the possibilities of hybridization with unreduced gametes would render the chart unmanageably complex. Whilst this clone bears a greater resemblance to R. sachalinensis than do any of the other inter-specific hybrids, its leaves are smaller than in true R. sachalinensis and it possesses hybrid hairs and epidermal characters (Plate 5.21). I propose that its greater resemblance to R. sachalinensis possibly stems from one of the following origins:



R. japonica var. *japonica* \times *Fallopia baldschuanica* $2n=54$

Details of the 11 locations where seed collected from octoploid *R. japonica* plants has been found to be of this combination, and the one plant found growing in the wild at Haringey, are shown in distribution map (Figure 5.7). It must also be stated at this point that I examined seed from 12 *R. japonica* parents in all and that only the plant at

Caerynch Hall (P5) did R. japonica produce anything but hybrids with F. baldschuanica. These locations are again mostly from Wales but spread as far north as Grange over Sands and as far south as Petersfield. I would also venture to say that all seed set in this country in male-sterile R. japonica plants isolated from Reynoutria pollen will be of this combination. An example of the ubiquity of this phenomenon may be gauged from the experience of Dr. R. Scott at Merlewood who collected seed from male-sterile R. japonica plants adjacent to R. sachalinensis plants, not unreasonably expecting them to be hybrids between the two Reynoutrias. Cytological examination at Leicester of the resulting plants showed them to be in fact hybrids with F. baldschuanica.

Description

Herbaceous perennial, younger plants weakly rhizomatous, but stout woody rhizomes in long-established plants; stems erect but bowing over, hollow, up to 2m long, slender with red blotches, less than 1cm diameter; leaves glabrous, slenderly petiolate (1.5-5cm), triangular ovate apex acuminate to acuminate cuspidate to 13x6.5cm, truncate at the base; inflorescence of axillary and terminal panicles. Flowers with 8 empty but well-developed anthers, stigma trifid slightly fimbriate, resembling in both size and morphology those of R. japonica rather than F. baldschuanica; Flowering September to early October, no reports of seed set.

R. sachalinensis x F. baldschuanica 2n=32

This combination, discovered subsequently to the R. japonica

var. japonica x F. baldschuanica hybrids, has not been pursued with such diligence since the R. japonica hybrids amply illustrate the true scale of the phenomenon. Only 5 R. sachalinensis clones were examined, and the 3 male-sterile ones produced only Fallopia hybrids (see distribution map Figure 5.7). The other two clones examined were actually hermaphrodites and produced a very low frequency of selfed seeds.

These plants show much more Fallopia characteristics than do those produced by octoploid Reynoutrias. This is presumably due to the different Reynoutria : Fallopia genome ratios 4:1 compared to 2:1.

Description

Woody perennial, some vertical rhizome growth but without any horizontal development; stems not twining, erect bowing right down to ground level at the tips, hollow initially but becoming woody up to about 75cm, lower regions lenticulate, with perennating buds at the nodes, up to 2m, 6-8mm diameter; Leaves glabrous apart from scattered short hairs on the veins of the lower epidermis, petiolate 3.5-4.5cm, shape triangular ovate to narrowly so, acuminate up to 14.5x8cm, strongly cordate base (sometimes sub sagittate) Inflorescence in terminal (mainly) and axillary panicles, flowers conspicuously winged in bud, resembling F. baldschuanica rather than R. sachalinensis, containing 8 well-developed anthers, and a trifid capitate-fimbriate stigma, flowering early October, no reports of seed production.

R. japonica var. compacta x R. sachalinensis x F. baldschuanica 2n=32

Surprisingly, since one parent is known only from 5 localities, this hybrid combination has been found as seed at two of these localities. These are, shown in distribution map Figure 5.7.

Description

A compact woody perennial, rhizomes not yet observed; stems erect, initially hollow but becoming woody along most of length, more branched than the R. sachalinensis hybrids, all woody portions strongly lenticulate, bearing perennating buds at nodes, up to 80cm long, diameter up to 8mm; leaves glabrous apart from scattered short hairs on the veins of the lower epidermis, slender petiolate (2.5-4.5cm); leaves ovate to triangular ovate, tips acuminate to acute, base cordate, to 6.5-11cm x 3-6.5cm. Inflorescence of terminal and axillary panicles (Plate 5.23), flower conspicuously winged in bud, resembling F. baldschuanica but smaller, 8 well-developed anthers and truly intermediate stigma (see Plate 5.23d), much more freely-flowering than the other Reynoutria x Fallopia hybrids, late September, early October, no recorded seed-set.

5.3.1_{ii} Artificial Hybrids

A number of artificial hybrids have been produced and examined morphologically and cytologically. These results indicate that the parentages assigned to these putative hybrids are correct. The artificial hybrids produced are as

follows:

- 1) R. sachalinensis (P57) x F. baldschuanica (P98) 2n=32

As very little endosperm was present embryo culture was used; 4 plants produced (P101a-d)

- 2) R. japonica var. compacta (P2b) x F. baldschuanica (P98)
2n=32

Only one plant (P69) produced via embryo culture. As the radicle was damaged during dissection it was necessary to use medium containing rooting hormone.

- 3) R. japonica var. japonica (P6) x F. baldschuanica (P182)
2n=54

3 plants produced via embryo culture (P94a-c).

- 4) R. sachalinensis x R. japonica var. compacta hybrids

These were produced reciprocally as follows:

R. sachalinensis(P57) x R. japonica var. compacta (P2b)
3 plants P79a-c 2n=44 By embryo culture

R. japonica var. compacta (P2a) x R. sachalinensis (P55)
2 plants P78a,b. 2n=44 By embryo culture.

- 5) R. japonica var. japonica x R. sachalinensis hybrids

R. japonica (P12) x R. sachalinensis (P55)

5 plants (P75a-e) via embryo culture 2n=66, except P75b
(2n=67)

- 6) R. japonica var. japonica (P12) x R. japonica var. compacta(P2b)

8 plants produced via embryo culture (P76a-h) 2n=66.

Reciprocal crosses involving the octoploid R. japonica could not be attempted as only female plants were known at that time. Attempts to make the inter-generic crosses

reciprocally, all failed and no seed was produced.

The frequency with which inter-generic hybrids between female Reynoutria clones and Fallopia baldschuanica occur in the wild and the ease with which they were synthesised artificially led naturally to a series of attempts at producing a) reciprocal hybrids with F. baldschuanica as the female parent, and b) crosses between Reynoutria species and other Fallopia taxa. The results of this research are shown in Figure 5.8. Immediately noticeable is the unilateral nature of the Reynoutria/Fallopia hybridization. Whilst hybrids were readily obtained between female Reynoutria and male Fallopia, I was unable to produce any inter-generic hybrids in which F. baldschuanica was the female parent. All attempts at Fallopia/Fallopia hybridizations and Reynoutria/Fallopia hybridizations failed except that between male-sterile R. sachalinensis and F. cilinodis. This has a question mark against it on Figure 5.8, since, although embryos with very little endosperm were produced, the radicle was damaged so that only the cotyledons developed and I was unable to produce a plant from it. Whilst the gynodioecious nature of the Reynoutria taxa allowed reciprocal hybridization without emasculation, the same was not true for the hermaphrodite flowers of Fallopia species. The annuals F. convolvulus and F. dumetorum provided great problems, since the anthers usually started to dehisce before the flower was ready to open, and, it was thus necessary to force the flowers open at a very young stage for emasculation—a process which usually led to the premature dropping of the flower. Hybrids between F.

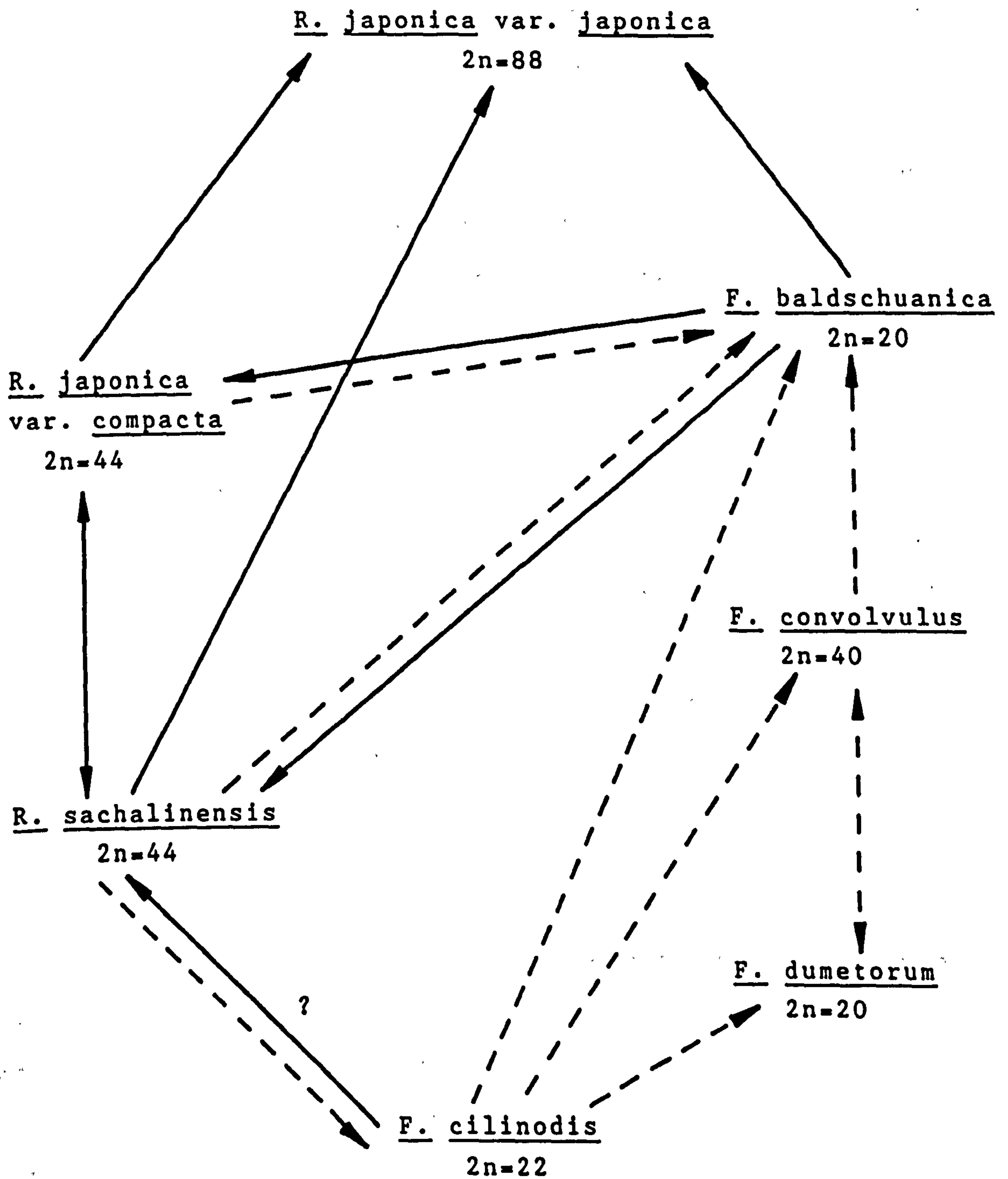


FIGURE 5.8 CHART SHOWING CROSSES ATTEMPTED BOTH WITHIN AND BETWEEN FALLOPIA AND REYNOUTRIA

convolvulus and F. dumetorum have been reported from the wild, F. x convolvuloides but these reports should be treated with caution since no clearly discontinuous differences separate the parents in the first place. Secondly, even if a male-sterile plant of either did arise, the inconspicuous flowers are unsuited for insect pollination and it is most unlikely that pollen of the other species would be transferred to it.

Since I suspected that my failure to produce hybrid plants might have more to do with the technical difficulties involved than the existence of intrinsic breeding barriers I did some in vitro pollination experiments between Fallopia and Reynoutria taxa. These results are presented in Table 5.1. They are necessarily qualitative, since it is impossible to assess the amount of pollen initially deposited on the stigma; neither can one account for loss of pollen during the various cleaning and washing stages of the technique. Nevertheless, some interesting points are revealed. The unreceptiveness of the F. baldschuanica stigma is highlighted, in contrast to the extremely receptive nature of the Reynoutria stigmas to F. cilinodis and F. dumetorum. Although penetration of the stigma by alien pollen grains does not automatically lead to fertilization, embryo production and germination, it is an essential first step and one at which the attempts to hybridize with F. baldschuanica fail. The penetration of Reynoutria stigmas by F. cilinodis pollen fits in well with the evidence already presented of R. sachalinensis x F. cilinodis seed production. Although, in the absence of

Stigma	Pollen	No. stigma Examined	Pollen attachment	Pollen germination	Pollen penetration	Comments
<u>F. baldsch.</u> P174	<u>F. dumetorum</u> P177b	7	+++	-	-	incompatibility reaction ? flowers emasculated in bud
<u>F. baldsch.</u> P174	<u>R. sachalinensis</u> x <u>R. jap. v. comp.</u> P79c	12	++	+	±	
<u>F. baldsch.</u> P163	<u>R. jap. v. comp.</u> P99c	6	+++	+++	-	
<u>R. jap. v. comp.</u> P2b	<u>F. dumetorum</u> P177c	6	+++	+++	+++	
<u>R. jap. v. comp.</u> P2b	<u>F. cilinodis</u> P148	6	+++	+++	+++	
<u>R. sachalinensis</u> P57	<u>F. cilinodis</u> P148	13	+++	±	±	some good penetration
<u>R. sachalinensis</u> P171	<u>F. dumetorum</u> P177b	11	++	±	±	

TABLE 5.1 POLLEN GERMINATION AND GROWTH IN INTERGENERIC POLLINATIONS

cytological investigation of such a plant, the remote chance of an alien pollen tube triggering apomictic seed production cannot be excluded.

5.3.2 MORPHOLOGY OF HYBRIDS

Reynoutria x Fallopia Hybrids

It may be recalled from Chapter 2 that R. japonica and F. baldschuanica are rather similar in terms of trichome types and cuticular ornamentation. The only trichomes found (type A) Plates 2.1c,d and 2.6d are little more than slightly swollen epidermal cells, and the cuticle generally lacks ornamentation except around the stomata and occasionally adjacent cells. It is then not surprising that hybrids between octoploid R. japonica and F. baldschuanica do not differ much from this (Plate 5.18). However, in some of these pentaploid hybrids, such as the artificial one (Plate 5.18b), there does appear to be more surface striation than is found in either parent.

The position of the tetraploid Reynoutria x Fallopia hybrids presents more interest with the exception of R. japonica var. compacta x F. baldschuanica (not illustrated) which is again similar to its parents. P102 which came from open pollinated seed from a putative R. japonica x R. sachalinensis hybrid, and is hence a triple hybrid, has some interesting trichomes which are also distributed in a singular manner. P33 from Gomshall, the female parent of P102, has trichomes similar to those in Plate 5.17a,b which





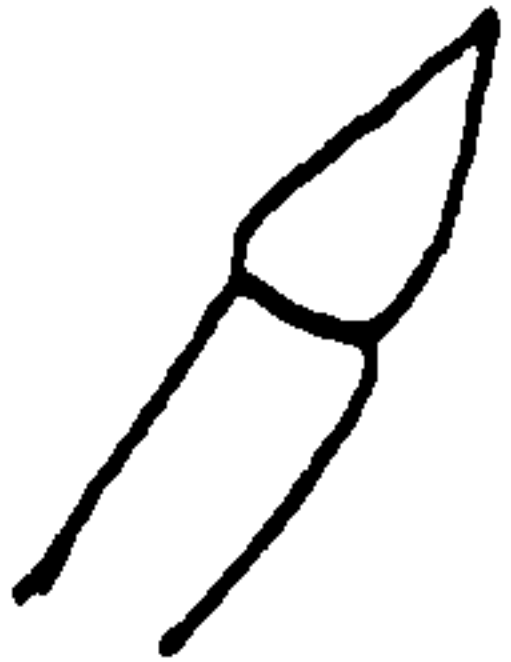
Accession				
<u>R. sach.</u> x <u>F. baldsch.</u> P140		.076 x .095	0.069	
4x <u>Reynoutria</u> x <u>F. baldschuanica</u> P102b	+	.056 x .1	+	
<u>R. sach.</u> x <u>R. jap.v.comp.</u> P79a		.057	.095-.19	
<u>R. sach</u> x <u>R. jap.v.comp.</u> P79c		.04	to .13	
<u>R. jap.v.comp.</u> x <u>R.sach.</u> P78a		.095	.057-.25	
<u>R. jap.v.comp.</u> x <u>R.sach.</u> P13	+	+	to .18	
<u>R. jap.v.jap.</u> x <u>F. baldschuanica</u> P80a	+			
<u>R. jap. var. japonica</u> x <u>F. baldschuanica</u> P80d	+			
<u>R. japonica var. japonica</u> x <u>F. baldsch. (art.)</u> P94	+			
<u>R. jap.v.jap.x R.sach.</u> P75c	+		+	
<u>R. jap.v.jap.x R.sach.</u> P75b	+	+	.095 to .18	
<u>R. jap.v.jap.x R.sach.</u> P75d	+	+	.095 to .19	
<u>R. jap.v.jap.x R.sach.</u> P31	+	to .04	to .13	
<u>R. jap.v.jap.x R. sach.</u> P32	+	+	to .17	
<u>R. jap.v.jap.x R.sach.</u> P146	+	+	to .18	
<u>R. jap.v.jap.x R.sach.</u> P52	+		+	
<u>R. jap. var. 'spectabile'</u>	+	+		
<u>R. japonica var. japonica</u> x <u>R. jap. var. comp.</u> P76b	+	to .063		
<u>R. japonica var. japonica</u> x <u>R. jap. var. comp.</u> P76a	+			
<u>R. jap. x R. sach.</u> P51B	+		.08 to .18	

TABLE 5.2 DISTRIBUTION OF TRICHOME TYPES

			Epidermis
	0.44		cuticle undulate, fine striae around stoma
	5 cell .45mm 5 cell (0.39mm) to 0.26 4 cell (0.73mm)	3 cell .18-.3mm 5 cell (.46mm) to 0.28	moderately to strongly striate (++) cell outline visible moderately to strongly striate (++) cell outline visible moderately to strongly striate (++) cell outline visible + striate
			± striate ± striate ± striate overall, + around stoma
	3 cell .16-.25 3 cell (.21mm) 4 cell (.39mm) 4 cell (.39mm) 2 cell (.25) 4 cell (.11-.37)	2 cell (.22mm)	± to + striate + striate + striate ± to + striate + striate + striate ± to + striate few striae, mostly around stoma
			± striae around stoma no striae
	to .4mm		+ to ++ striate

AND CUTICLE STRIATIONS

are intermediate between those of R. japonica and R. sachalinensis. The hairs of P102 (Plate 5.15b,c) represent an intermediate structure between those of the parents, but seem to be distributed in groups rather like the type A trichomes of the Fallopia parent, and unlike the scattered distribution of hairs of the R. japonica x R. sachalinensis hybrids. The cuticular striations of P102 resemble those of the Fallopia parent rather than those of the Reynoutria parent and may thus reflect the greater relative dose of Fallopia than is to be found at the pentaploid level.

Hybrids between R. sachalinensis and F. baldschuanica are strikingly intermediate between the highly striate R. sachalinensis cuticle with long slender uniseriate hairs and the smooth Fallopia cuticle with small rounded trichomes (Plate 5.14.2). These trichomes appear to be identical to those found in R. japonica x R. sachalinensis hybrids (compare Plate 5.14.2f with 5.16b) Plate 5.14.2e shows the fine reticulate striations found in these hybrids, a pattern that more closely resembles F. cilinodis than the R. sachalinensis x R. japonica hybrids. Hybrid trichome and cuticle types are summarized in Table 5.2.

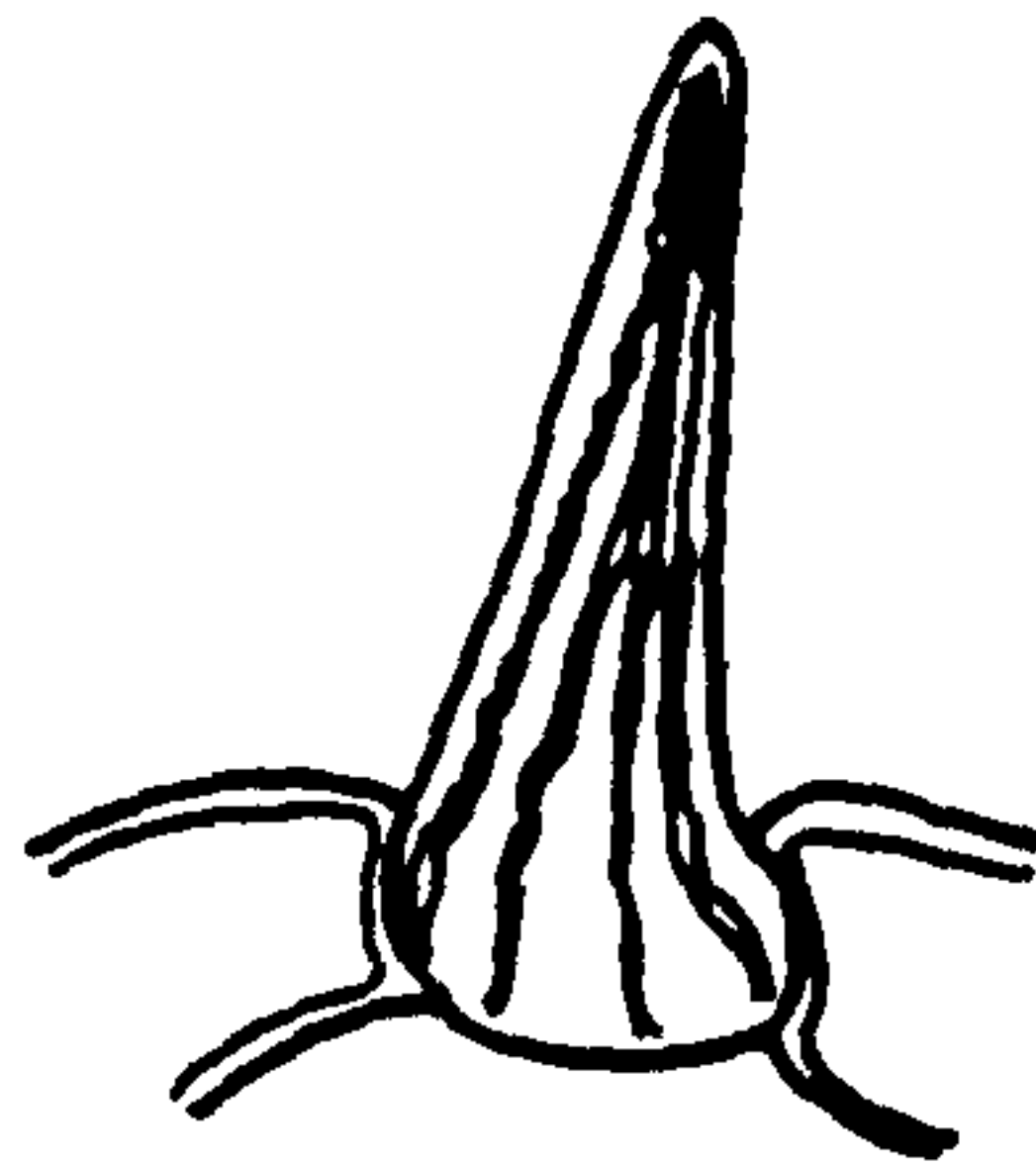
Inter- and Intra-specific Reynoutria hybrids

The intra-specific hybrid R. japonica var. japonica x R. japonica var. compacta (Plate 5.20 b-f) has not surprisingly the type A trichomes and the smooth barely striate cuticle which characterises both of its parents. R. japonica x R. sachalinensis is found at three ploidy levels (4x, 6x and

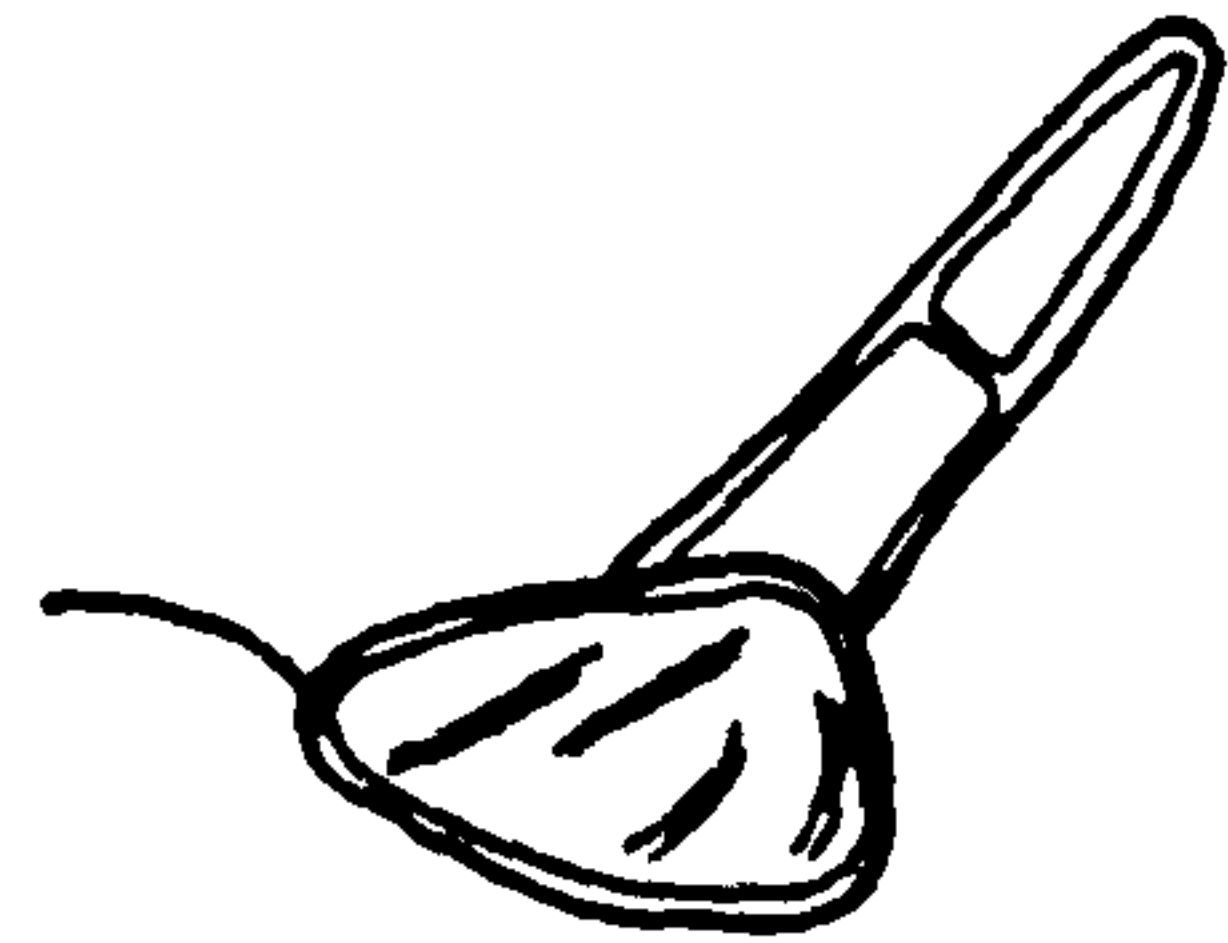
Type A
R.japonica



Hybrid hairs

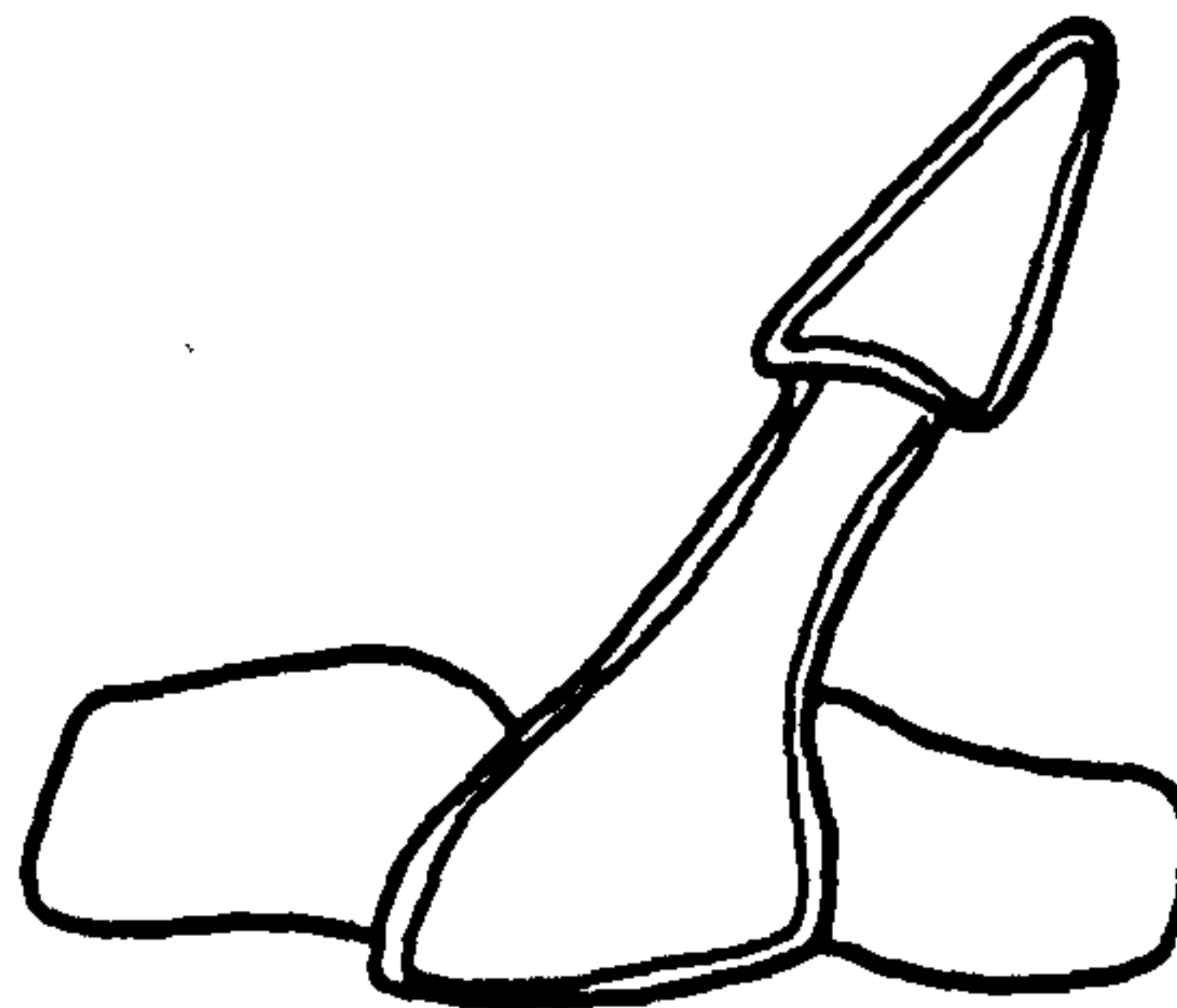


Type D



Type E

Type F



Type B
R.sachalinensis

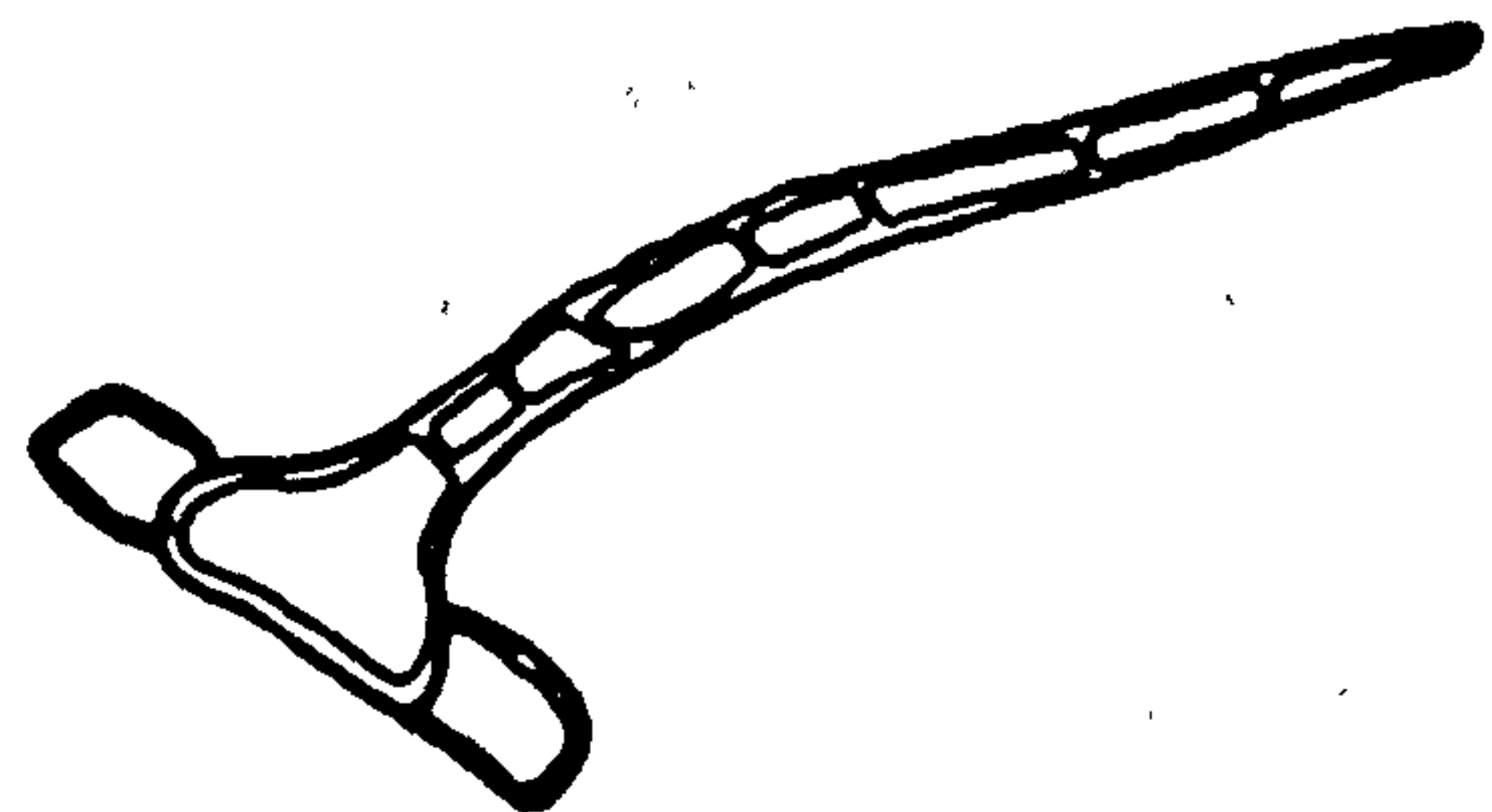


Figure 5.9 Hybrid *Reynoutria* trichomes

8x). In Britain the tetraploid hybrid probably represents a cross between R. japonica var. compacta and R. sachalinensis. A hybrid between a tetraploid R. japonica var. japonica such as P114b and R. sachalinensis would be extremely similar in terms of trichome and epidermal structure but would probably be a larger plant. The lack of 4x R. japonica var. japonica in the British Isles suggests that they have not been involved in hybridization with R. sachalinensis over here.

Figure 5.9 shows both parental and hybrid hairs found in these R. japonica x R. sachalinensis hybrids, hybrid hairs designated D to F. Type D is the simplest type (Plates 5.16a, 5.17b), is unicellular striate and tapers to a fine point. Type E consists of one or two heavily striate oblong basal cells plus one or more sharply tapering cells and is found in these hybrids at all three ploidy levels. Type F a multicellular uniseriate hair is similar to E, except that successive cells have a base broader than the apex of the cell to which they adjoin giving the curiously shaped structures shown in Plate 5.16c. It has not been possible to reliably separate the tetraploid and hexaploid hybrids on the basis of trichome types. The octoploid R. japonica x R. sachalinensis hybrid (Plate 5.21b and c) has similar hairs to the tetraploid and hexaploid though somewhat larger.

The degree of epidermal striation is again intermediate between that of the parents and is a characteristic feature of R. japonica x R. sachalinensis hybrids. It will be noted that there is a good correspondence between the epidermal

ornamentation and trichome types between the artificial and putative hybrids. These characters are particularly useful as, given that one of the larger basal leaves is present, the hybrid may be identified from herbarium material. The hybrid hairs are of particular value since it is almost impossible to confuse them with the small striate protruberances of R. japonica or the long multicellular barely striate R. sachalinensis trichomes.

5.3.3 CYTOLOGY OF HYBRIDS

R. japonica var. compacta x F. baldschuanica 2n=32

This is known only from a single plant produced via embryo culture at Leicester. The mitotic complement is shown in Plate 5.1d. The karyotype is much more asymmetrical than in the Reynoutria species (see Plates 3.3 3.4), due to the presence of the ten larger Fallopia chromosomes. As this plant has yet to flower I have not been able to examine its meiosis, though as it is a triploid one would anticipate it to be most irregular. The 2C value has been determined as 2.6pg DNA or 1.73pg per 2x genome.

R. sachalinensis x F. baldschuanica 2n=32

This has been found in several locations as seed, but no plants of it have been found in the wild; artificial hybrids have also been produced. Plate 5.1a,b shows the mitotic complement and Figure 5.1 the karyotype and karyogram. The larger Fallopia chromosomes are particularly evident in

these hybrids. These plants have occasionally produced flowers and accordingly meiotic figures are shown in Plate 5.6 and the results of meiotic analysis in Tables 5.3 and 5.4. These plants have some of the most irregular of meiosis that I have ever seen. The low number of bivalents or trivalents produced (means of 4.8 and 0.71 respectively) causes there to be no proper spindle formed and consequently the stages between late diakinesis and telophase are almost impossible to distinguish with certainty. Plates 5.6a,b,c illustrate this well, showing the typical pre-telophase I appearance with chromosome bodies more or less scattered randomly throughout the cytoplasm. Laggards and bridges are often found at late telophase I (Plate 5.6d) and, whilst there is sometimes a second telophase (Plate 5.6f), meiosis often aborts at telophase I giving restitution diads with micronuclei (Plate 5.6e). The 2C DNA value (Table 5.5) was determined as 2.7pg or 1.8pg per 2x genome.

R. japonica var. japonica x F. baldschuanica 2n=54

This hybrid is generally found when seed from R. japonica var. japonica plants (growing in the absence of Reynoutria pollen) is examined; further a single well established plant was recently discovered in London (Bailey 1988). The distinctive chromosome complement is shown in Plates 5.3a,b where both artificial and putative hybrids are represented, the karyotype and karyogram in Figure 5.2, and the meiotic behaviour in Plate 5.9. This hybrid does not flower with any regularity so I have had only limited opportunities to examine its meiosis. I have been able to analyse only 5

Taxon	Chr. No.	No. Cells Examined			
		Chr. Bodies	Bivalent/ multivalent	Chiasma frequency	
4x <u>Reynoutria</u> x <u>F. baldsch.</u> P102a	32		10		
4x <u>Reynoutria</u> x <u>F. baldsch.</u> P102b	32		5		
<u>R. sach.</u> x <u>F. baldsch.</u> P101b	32		7		
<u>R. jap.v.comp.</u> x <u>R. sach.</u> P13	44		10	10	
<u>R. jap.v.comp.</u> x <u>R. sach.</u> P78a	44		3	2	
<u>R. sach.</u> x <u>R. jap.</u> <u>v.comp.</u> P79c	44		34	26	
<u>R. jap.x F. baldsch.</u> P80d, P83b, P157b	54	16	5		
<u>R. jap.v.jap.x</u> <u>R. sach.</u> P29	66	2			
<u>R. jap.v.jap.x</u> <u>R. sach.</u> P75d	66	7	3		
<u>R. jap.v. jap. x</u> <u>R.jap.v. comp.</u> P76b	66	3	3		
<u>R.jap.x R.sach.</u> P119	66	7			
<u>R.jap.x R.sach.</u> P32	66	11	9		
<u>R. jap.x R. sach.</u> 8x P51b	88		12	3	

TABLE 5.3 HYBRID MEIOTIC CONFIGURATIONS

The total number of cells examined is the sum of the first three columns.

Meiotic configurations									
	I	Rod IIs	Ring IIs	Total IIs	IIIs	3 xta IVs	4 xta IVs	5 xta IVs	VI
	189			61	2	1			
	46			28	17	2			
	141			34	5				
		163	40	203		5	1	1	
		41	3	66					
	5	422	96	645	1	19	5	1	1
	50			110					
	52			62	7				
	40			39	11	4			
	153			169	23	6			
	4	92	20	465		22	5		1

Taxon	Mean Meiotic Configurations										Chr. No.	Mean xta. per II equiv.	Mean No. chr. bodies
	I	Rod II	Ring II	Aggr. IIs	IIIs 3xta. IV		4xta. IV	5xta. IV	VI				
4x Reynoutria x F. baldsch. P102a	18.9			6.2	0.2	0.1				32		25.4	
4x Reynoutria x F. baldsch P102b	9.2			5.6	3.4	0.4				32		18.6	
R. sach. x F. baldsch. P101b	20.1			4.8	0.71					32		25.6	
R. jap v. comp. x R. sach. P13		16.3	4.0			0.5	0.1	0.1		44	1.21	21	
R. jap v. comp. x R. sach. P78a		20.5	1.5							44	1.07	22	
R. sach. x R. jap. v. comp. P79c	0.2	16.2	3.7		0.04	0.76	0.2	0.04	0.04	44	1.35	21.3	
R. jap. x F. baldsch. P80d, P83b, P157b	10			22						54		32.4	
R. jap. v. jap. x R. sach. P29												35.5	
R. jap. v. jap. x R. sach. P75d	17.3			20.66	2.33					66		38.3	
R. jap. v. jap. x R. jap v. comp. P76b	13.3			13	3.66	1.33				66		33.7	
R. jap. v. jap. x R. sach. P119												40.6	
R. jap. v. jap. x R. sach. P32	17			18.8	2.55	0.66				66		39.6	
8x R. jap. x R. sach. P51b	0.3			38.75		1.83	0.42		0.08	88	1.21	41.4	

TABLE 5.4 MEAN HYBRID MEIOTIC CONFIGURATIONS

Taxon	Machine Units			Picogram DNA		Chromosome Number
	Mean 2C	S.D.	no. cells	2C DNA	DNA per 2x genome	
<u>R. jap. v. comp. x F. baldsch. P69</u>	27.82	4.25	77	2.6	1.73	32
<u>R. sach. x F. baldsch. P145</u>	33.4	4.3	30	2.7	1.8	32
<u>R. jap. v. comp. x R. sach. P78a</u>	29.6	2.86	93	2.92	1.46	44
<u>Reynoutria hybrid P85</u>	39.17	3.79	61	3.66	1.67	55
<u>R. japonica x F. baldschuanica P164</u>	52.41	5.63	75	4.22	1.68	54
<u>R. japonica x R. sachalinensis P32</u>	48.38	4.53	80	4.78	1.59	66
<u>R. japonica x R. sachalinensis P75c</u>	39.84	4.9	55	3.93	1.31	66
<u>R.jap. v.jap. x R.jap. v. comp P76a</u>	37.52	3.76	46	3.7	1.23	66
<u>R. jap. x R. sach. 8x P51b</u>	52.6	7.33	63	5.19	1.3	88

TABLE 5.5 FEULGEN MICRODENSITOMETRY RESULTS

cells on a bivalent univalent basis, but larger numbers of polar views of metaphase I on a chromosome body basis. Plate 5.9e is a good example of the former and clearly shows that a good deal of bivalent formation is taking place. Plate 5.9d is one of the polar metaphases which always seem to have 32 chromosome bodies. All my analyses show that 22 bivalents and 10 univalents are regularly produced in these hybrids, the simplest explanation being that the 44 Reynoutria chromosomes are pairing autosyndetically and the 10 Fallopia ones are left as univalents. Plate 5.10a shows a metaphase I with a tight spindle and 10 univalents, presumably the Fallopia chromosomes. These hybrids have a very low pollen fertility; Plate 5.10 shows why this is so. Plate 5.10b shows a number of chromosomes left on the spindle at telophase I and Plate 5.10c shows late telophase with two micronuclei which have been formed presumably from chromosomes left on the spindle. Plate 5.10e shows a telophase II with a number of micronuclei, probably the result of division of Telophase I micronuclei. Plate 5.10f shows the pollen tetrad stage with a number of micronuclei. The 2C DNA value was determined as 4.22pg or 1.68pg per 2x genome equivalent.

R. japonica x R. sachalinensis x 4x Reynoutria 2n= C55

Although this particular combination has been omitted for reasons of clarity from Figure 5.3 it is nonetheless of some interest. Plate 5.3c shows the metaphase configuration of one such individual with 55 chromosomes and a telocentric fragment. This Plate may usefully be compared with Plates

5.3a and b, which show the pentaploid hybrids with F. baldschuanica. It may be distinguished from these by its much more symmetrical karyotype. The only plant to flower, P108a managed the remarkable feat of producing subterranean flowers in its plant pot, which must cast some doubt on the reproductive competence of such hybrids.

The 2C DNA value of P85a was determined as 3.66pg or 1.67pg per 2x genome equivalent.

R. japonica var. compacta x R. sachalinensis 2n=44

Plate 5.2 shows the metaphase complement of two artificial hybrids and one wild hybrid. As the karyotypes of the two parents are virtually identical, I have not attempted to produce a karyotype of this hybrid. Meiosis is remarkably regular overall, one accession P78a producing only bivalents (see Plate 5.7e,f), this being one of the very few examples of clearly recognisable meiotic figures. Accessions P13 and p79c often have a quadrivalent present, and, judging by the lack of trivalents and univalents in these hybrids, is probably due to their being heterozygous for a non-homologous reciprocal translocation. Where it has been possible to analyse cells for chiasmata frequency, rod bivalents have been found to be much more common than ring bivalents (Table 5.4). The chiasma frequency of between 23.5 and 29.8 does not differ significantly from those of R. japonica var. compacta, tetraploid R. japonica var. japonica and R. sachalinensis (Table 3.5). The 2C DNA value of 2.92pg lies between the values recorded for the two parental

species (Table 3.2).

R. japonica var. japonica x R. japonica var. compacta 2n=66

In Britain this combination is known only from artificial hybrids but I have seen plants in Aachen (Germany) which closely resemble them. Plate 5.4b shows the 66 metacentric to submetacentric chromosomes, whilst meiosis is illustrated in Plate 5.11. This meiosis is extremely difficult to interpret, and the results must be regarded as somewhat tentative. From the small number of cells analysed it can be seen that multivalents are more frequent than in other Reynoutria hybrids and that the mean number of bivalents approximately equals the number of univalents at 13 per cell (Tables 5.2 and 5.3). The 2C DNA value of 3.7pg (Table 5.5) lies in between the values determined for the parental taxa.

R. japonica var. japonica x R. sachalinensis 2n=66

This is the most common Reynoutria hybrid growing in Britain (Figure 5.5), and naturally occurring seed of this parentage has been found at Brithdir near Dolgellau, where a male-fertile R. sachalinensis grows near to a male-sterile R. japonica. Artificial hybrids have been made at Leicester. Plate 5.4 shows mitotic preparations of two examples of natural hybrids. The chromosomes complement is the usual symmetrical Reynoutria complement of sub-metacentric and metacentrics. Meiotic figures are shown on Plates 5.12 and 5.13 and are extremely difficult to analyse on the grounds of their generally sticky nature and

the ill-defined shapes of the bivalents and multivalents even in good cells. I have tried to analyse these figures as objectively as possible, so that regrettably in some cases the sum of the meiotic figures does not equal the chromosome number. Multivalents are found (Plate 5.12e), usually trivalents, at a frequency of between 2.3 and 2.6 per cell. The mean number of chromosome bodies per meiocyte (Table 5.4) varies between 35.5 and 40.57 and is composed primarily of bivalents, univalents and a few trivalents. This irregular meiosis is in marked contrast to the regular bivalent formation of tetraploid R. japonica var. compacta x R. sachalinensis hybrids. 2C DNA values ranging between 3.9 and 4.8 have been determined (Table 5.5)

Octoploid R. japonica x R. sachalinensis 2n=88

This hybrid is known only from a single locality, a roadside near Dolgellau, where it grows next to a hexaploid male-sterile R. japonica x R. sachalinensis hybrid. At the time that I was undertaking this research this plant was the only male-fertile octoploid that I had, and is thus my only example of octoploid Reynoutria meiosis. I have not got a good mitotic preparation of this plant, but from what I have seen of it, it has the typical sub-metacentric to metacentric chromosomes that typify Reynoutria cytology.

I have obtained some good interpretable meiotic preparations and these are shown in Plate 5.14.1. Of the 12 cells that I was able to analyse 11 had one or more quadrivalents present; these are shown particularly well in Plate 5.14b.

Apart from these quadrivalents and the very occasional occurrence of univalents the meiosis is entirely regular with large numbers of bivalents present.

The origin of this plant is something of a mystery, since morphologically it is certainly intermediate, although the chromosome number suggests R. japonica. What is more, it seems closer morphologically to R. sachalinensis. The easiest means of producing such a hybrid would be by an unreduced gamete from R. sachalinensis pollinating an octoploid R. japonica. An alternative route being an R. sachalinensis back-cross to an unreduced gamete from a hexaploid R. japonica x R. sachalinensis hybrid. It is interesting to note that quadrivalents appear to be more common in the 4x and 8x ploidy levels than at the 6x ploidy level, although this could be a function of the more regular meiosis at these ploidy levels, the irregular 6x meiosis making it difficult to spot quadrivalents. The 2C DNA value per 2x genome fits into the range of values found in the tetraploid and hexaploid inter-specific Reynoutria hybrids.

5.3.4 HYBRID FERTILITY AND FECUNDITY

The ultimate significance of a new hybrid can be measured by the success it has in establishing itself and by the degree to which it allows gene flow between previously separate taxa or genera. In order to assess this potential it is necessary to examine the reproductive competence and longevity of hybrids. As to the latter, the inter- and intra-specific Reynoutria hybrids appear to be potentially

immortal. The single known plant of R. japonica x F. baldschuanica in the wild is long established and possesses a thick woody rhizome and gives every impression of being potentially very long-lived. The triploid intergeneric hybrids on the other hand appear to lack strong rhizome development and thus may be of more limited duration. This section deals mainly with the various methods of assessing reproductive competence such as pollen viability, pollen germination in-vitro and in-vivo, seed-set and seed germination.

5.3.4₁ Pollen Fertility

The most convenient assessment of fertility for male-fertile plants is an examination of the pollen. Pollen stainability with Münztings carmine is a widely used technique, and although it only measures the existence and regularity of the cytoplasm of the pollen grains it provides a useful yard-stick for comparative assessments. The only unequivocal measure of course is germination and fertilization of the female gamete. Pollen stainability results are shown in Table 5.6 along with the size range. It should be noted that only grains assessed as fertile were used for measuring purposes, which explains the lack of measurements for those accessions with a very low fertility. In my assessment of pollen fertility I have used fairly strict criteria, only scoring those grains that were completely filled with cytoplasm. This was necessary as there was no very clear cut-off between fertile and infertile since the vast majority of grains even in the

Taxon		POLLEN SIZE	
		1.0-1.2	1.3-1.5
<u>F. baldschuanica</u>	P151	38	13
<u>F. baldschuanica</u>	P152	8	94
<u>F. baldschuanica</u>	P163	119	54
<u>F. baldschuanica</u>	P174	40	53
<u>F. cilinodis</u>	P148	66	31
<u>F. convolvulus</u>			8
<u>R. japonica</u> var. <u>compacta</u>	P2a	72	33
<u>R. japonica</u> var. <u>compacta</u>	P99		59
<u>R. japonica</u> var. <u>compacta</u>	P173	2	49
<u>R. sachalinensis</u>	P68	2	35
<u>R. sachalinensis</u>	P55	3	43
<u>R. sachalinensis</u>	P62	4	51
<u>R. sachalinensis</u>	P180	46	60
<u>R. japonica</u> 4x	P114	2	41
N. <u>R. jap. v. comp.</u> x <u>R. sach.</u>	P13	8	77
A. <u>R. jap. v. comp.</u> x <u>R. sach.</u>	P78a	8	73
A. <u>R. sach.</u> x <u>R. jap. v. comp.</u>	P79c	31	12
<u>R. jap.</u> x <u>R. jap. v. comp.</u>	P76a		
<u>R. jap.</u> x <u>R. jap. v. comp.</u>	P76b		
A. <u>R. japonica</u> x <u>R. sachalinensis</u>	P75d	13	63
N. <u>R. japonica</u> x <u>R. sachalinensis</u>	P45		
N. <u>R. japonica</u> x <u>R. sachalinensis</u>	P32	4	60
N. <u>R. japonica</u> x <u>R. sachalinensis</u>	P119		
N. <u>R. japonica</u> x <u>R. sachalinensis</u>	P136b	5	79
N. <u>R. japonica</u> x <u>R. sachalinensis</u>	P51B		25
(<u>R. jap. v. comp.</u> x <u>R. sach</u>) x			
<u>F. baldschuanica</u>	P102a, b		
<u>R. sachalinensis</u> x <u>F. baldschuanica</u>	P140a		
<u>R. sachalinensis</u> x <u>F. baldschuanica</u>	P101a		
<u>R. jap. var. jap.</u> x <u>F. baldsch.</u>	P80d		
<u>R. jap. var. jap.</u> x <u>F. baldsch.</u>	P157		
<u>R. jap. var. jap.</u> x <u>F. baldsch.</u>	P164		

TABLE 5.6 POLLEN SIZE AND STAINABILITY

E.P.U.s			No. Grains	% Fertility	Comments
	1.6-1.8	1.9-2.2			
			336	11.9	2x species
			316	78.5	
			618	87.8	
			322	49.1	
			311	95.2	
	29		190	100	4x species
			316	98.7	
			340	55.3	
	2		279	88.5	
	70		315	62.9	
			310	71	
	50	3	306	58.8	
			346	52.6	
	6		341	66.6	
	12	9	300	77.7	4x hybrids
	23		303	44.9	
			369	59.6	
			308	26.6	6x hybrids
			284	8.4	
	24		333	28.5	
			1134	6.3	
	8	9	307	22.8	
			321	11.5	
	16	1	349	55.6	8x hybrid
	65	6	326	68.4	
				0	3x & 5x hybrids
				0	
		18	325	7	
			210	0	
			462	2.6	
			87	0	

OF SPECIES AND HYBRIDS

triploids and pentaploids had some cytoplasm present.

The first point of interest is that there is no obvious increase in the pollen diameter as the ploidy level increases to the hexaploid level most being between 25 and 29µm. The diploid taxa are all capable of attaining very high levels of fertility, although it should be noted that there is considerable variation between the various accessions of F. baldschuanica, P151 having only a 12% fertility. Moderately high to high fertilities are recorded for the tetraploid species although two R. sachalinensis accessions are below 60%. The tetraploid Reynoutria pollen stainability is within the range found for the tetraploid species and indicative of regular meiosis. There is a dramatic drop in fertility when the hexaploid Reynoutria hybrids are considered, all but one being in the range of 8 to 29%. At the octoploid level the one plant has a reasonably high fertility comparable with the Reynoutria species and has pollen of larger size than the other taxa examined. As is to be expected at the 3x and 5x ploidy levels extremely low fertilities were recorded <7%.

5.3.4 In vitro pollen germination

Polygonum pollen is tri-nucleate when shed and (apart from the notable exception of grasses) it is generally impossible to germinate this in artificial media without the presence of stigmas or stigmatal extracts. Consequently, in order to get a quantitative assessment of pollen fertility, a technique was designed which allowed pollen to be in the

Pollen		Sucrose concentration		P. number	with various stigmas			without stigmas		with stigmas
		15%	17.5%		20%	15%	20%	MEAN%		
Species										
F.baldschuanica		P174	4.5		6.49	3.13	0	5.49		
R. japonica var. compacta		P99a	27.65		44.67			36.16		
R. sachalinensis		P68	13.5		18.2			15.85		
R. sachalinensis		P55	29.8			6.69		29.8		
R. sachalinensis		P62	26.6	23.11	12.37	3.7	1.62	20.7		
4x hybrids										
Putative 4x R.japonica x R.sachalinensis		P13	4.38	5.16	1.96			3.8		
Art. R.sach. x R.jap. var. compacta		P79c	6.1	12.15				9.13		
Art. R.jap. var. compacta x R. sach.		P78a	15.8	8.47	8.9			11.1		
6x hybrids										
Art. R.jap. x R.jap. var. compacta		P76a	0		1.17			0.59		
Art. R.jap. x R.jap. var. compacta		P76b	0.71	0	1.44			0.72		
Artificial R.japonica x R.sachalinensis		P75d	2.6	0	0.24			0.95		
Putative R.japonica x R.sachalinensis		P32		12.3				12.3		
Putative R.japonica x R.sachalinensis		P119	0		0			0		
8x hybrids										
Putative R.japonica x R.sachalinensis		P50	33.3		21.34			27.32		
Putative R.japonica x R.sachalinensis		P51b	21.05	12.31	13.69			15.7		

TABLE 5.7 IN VITRO POLLEN GERMINATION (STIGMA VARIATION AVERAGED)

Stigma Pollen	P. No.	v/o stigma	R.jap. v. comp. P2b	R.sach. P60	R.sach. P57	R.sach. P61	Put. R.jap. x R.sach. P31	Put. R.jap. x R. sach. P17	Mixed R.jap. & R.sach.
F.baldsch.	P174	3.125				0.12		7.7	
R.jap. v.comp.	P99a					36.2			
R.sach.	P68		23			14.63	23.8	2.09	
R.sach.	P55	6.69			29.8				
R.sach.	P62	2.66						19.5	23.11
4x hybrids	P13					3.17			5.16
	P79c						6.1		12.2
	P78a			14.9	9.6			12.6	8.47
6x hybrids	P76a						0.59		0
	P76b						1.1		0
	P75d			1.45					12.31
	P32					0			
	P119								
8x hybrids	P50		24.9					9.85	12.31
	P51b							27.32	

TABLE 5.8 IN VITRO POLLEN GERMINATION (SUCROSE CONCENTRATIONS AVERAGED)

presence of appropriate stigmas on a cellophane film which could then be viewed under the microscope (see Material and Methods). Since in the course of these experiments both the sucrose concentration and the stigmas or combinations of stigmas were independently varied, I have presented the data in two tables; Table 5.7, in which the stigma variation has been averaged; and Table 5.8, in which stigmas are listed, but the sucrose concentration results are averaged. By way of a control I did actually try to germinate some Reynoutria and Fallopia pollen in the absence of stigmas and obtained germination rates of between 2.7 and 6.7% (Table 5.8). Whilst in a few cases this rate exceeded that of pollen grown in the presence of appropriate stigmas, pollen germination in the absence of stigmas was between 12 and 50% of the maxima obtained using stigmas. The highest germination rate obtained was 44.7% obtained for R. japonica var. compacta, and 30% the highest found for R. sachalinensis. I am not in a position to say whether these represent the maximum germination rates possible or to what extent factors such as the age of the pollen, the time of day, or the physiological state of the plants have on the germinability of the pollen. Suffice to say the means shown in Table 5.7 follow the same sort of pattern that was found with the pollen stainability (Table 5.6), with the proviso that the gap in percentage terms between the tetraploid species and the hexaploid hybrids is considerably larger with pollen germination, and also that the tetraploid Reynoutria hybrids show distinctly less fertility in germination terms. The octoploid hybrid is again of comparable fertility with the tetraploid species. The F.

baldschuanica plant examined had a very low germinatability, in contrast to a 50% fertility revealed by Müntzings carmine. PLate 5.22d shows germinated pollen of the octoploid Reynoutria hybrid P51b.

5.3.4_{iii} In vivo pollen germination

These experiments involve the in vivo germination of pollen on in vitro flowers on agar. This was embarked upon in an attempt to answer three questions: how effective is hybrid Reynoutria pollen at germination and penetration of the stigma; how receptive are the stigmas of male-fertile plants; and is there any self-incompatibility reaction with hermaphrodite plants? The results are presented in Tables 5.9 and 5.10, and contain qualitative assessments of pollen adhesion, pollen germination and pollen growth in the stigma and style. I am not sure if there is any scientific basis for the pollen adhesion category, but it is a fact that on some stigmas the pollen deposited does not survive the cleaning and staining procedure, and in any case it is essential to record this since good germination and penetration cannot occur if hardly any pollen is attached to the stigmas.

The examination of pollen germination on the male-fertile plants (Table 5.9) presented some technical difficulties, as emasculation tended to adversely affect the development of the flowers. In order to try and overcome this difficulty unemasculated flowers were used and the results obtained with flowers that had been additionally cross-pollinated

Stigma	Pollen
<u>F. baldschuanica</u> P163	<u>R. japonica</u> var. <u>compacta</u> P99c
Art. <u>R. jap.</u> var. <u>comp.</u> x <u>R. sachalinensis</u> P78a	<u>R. japonica</u> var. <u>compacta</u> P173
Art. <u>R. jap.</u> var. <u>comp.</u> x <u>R. sachalinensis</u> P78a	<u>R. sachalinensis</u> P68
Art. <u>R. jap.</u> var. <u>comp.</u> x <u>R. sachalinensis</u> P78a	<u>R. sachalinensis</u> P62
<u>R. sachalinensis</u> P55	Put. <u>R. jap.</u> x <u>R. sach.</u> 8x P51B
<u>R. sachalinensis</u> P55	<u>R. sachalinensis</u> P68
<u>F. baldschuanica</u> P174	Art. <u>R. sach.</u> x <u>R. jap.</u> v. <u>comp.</u> P79c
<u>F. baldschuanica</u> P174	<u>F. dumetorum</u> P177
<u>R. sachalinensis</u> P62	<u>R. japonica</u> var. <u>compacta</u> P173
Put. <u>R. jap.</u> x <u>R. sach.</u> 8x P51B	<u>R. sachalinensis</u> P62
Put. <u>R. jap.</u> x <u>R. sach.</u> 8x P51B	<u>R. japonica</u> var. <u>compacta</u> P173
<u>R. sachalinensis</u> P68	Art. <u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u> P78a
<u>R. sachalinensis</u> P68	hybrid 8x P51B

Art. = artificial

Put. = putative

TABLE 5.9 POLLEN GERMINATION AND GROWTH ON THE

	No. stigmas exam.	Pollen attachment	Pollen germin.	Pollen* penetrat.	Comments
	6	+++	+++	-	stigmas not fully mature
	9	+	±	(-)±	
	12	±	±	(-)±	stigmas not well developed
	10	±	±	(-)±	some stigmas immature
	14	+++	+++	(-)+++	
	6	+++	+++	±	stigmas mature
	12	++	++	±	?incompatability reaction
	7	+++	-	-	
	6	+++	+++	(±)+++	
	10	+	+	(±)±	4 flowers poor stigma development
	6	+++	+++	(±)+++	
	5	+++	+++	(±)++	
	6	++	++	(±)++	some tubes branched and swollen

* figures in brackets relate to the results of a control set of selfed flowers.

STIGMAS OF MALE-FERTILE PLANTS

Stigma	Pollen	
<u>R. jap.</u> P179	Art. <u>R. sach.</u> x <u>R. jap.v.comp.</u>	P79c 4x
<u>R. jap.</u> P179	Art. <u>R. jap.v.comp.</u> x <u>R.sach.</u>	P78a 4x
<u>R. jap.</u> P12	Art. <u>R. jap.v.comp.</u> x <u>R. sach.</u>	P78a 4x
<u>R. jap.v.comp.</u>	<u>R. jap.</u> x <u>R. jap. v. comp.</u>	P76b 6x
<u>R. jap.</u> P179	<u>R. jap.</u> x <u>R. jap. v. comp.</u>	P76a 6x
<u>R. jap.</u> P179	<u>R. jap.</u> x <u>R. jap. v. comp.</u>	P76b 6x
<u>R. jap.</u> P12	<u>R. jap.</u> x <u>R. jap. v. comp.</u>	P76a 6x
<u>R. jap.</u> P12	Put. <u>R. jap.</u> x <u>R. sach.</u>	P119 6x
<u>R. jap.</u> P179	Put. <u>R. jap.</u> x <u>R. sach.</u>	P119 6x
<u>R. jap. v.comp.</u>	Put. <u>R. jap.</u> x <u>R. sach.</u>	P32 6x
<u>R. jap.</u> P179	Put. <u>R. jap.</u> x <u>R. sach.</u>	P32 6x
<u>R. jap.</u> P179	Art. <u>R. jap.</u> x <u>R. sach.</u>	P75d 6x
<u>R. jap. v.comp.</u>	Art. <u>R. jap.</u> x <u>R. sach.</u>	P75d 6x
<u>R. jap.</u> P179	Put. <u>R. jap.</u> x <u>R. sach.</u>	P51b 8x
<u>R. jap.</u> P12	Put. <u>R. jap.</u> x <u>R. sach.</u>	P51b 8x
<u>R. jap. v.comp.</u>	Put. <u>R. jap.</u> x <u>R. sach.</u>	P50 8x
<u>R. jap.</u> P179	Put. <u>R. jap.</u> x <u>R. sach.</u>	P50 8x

TABLE 5.10 PERFORMANCE OF HYBRID POLLEN ON FEMALE REYNOUTRIA STIGMAS

compared with a group of self-pollinated flowers collected at the same time. The figures for the self-pollinated controls are given in brackets, and in no case even equals the pollen penetration rate of the non-selfed pollen. Fuller details of the performance of selfed stigmas in male-fertile plants are given in Table 4.2 and show that little pollen germination or penetration occurs and that that which does is often accompanied with an incompatibility reaction. Apart from F. baldschuanica, the stigmata of male-fertile plants appear to be fairly amenable to penetration by non-self Reynoutria pollen (Table 5.9)

Table 5.10 shows the performance of hybrid Reynoutria pollen back-crossed to one or other parent. These results are generally in line with the in vivo pollen germination results, although the vigour of the octoploid hybrid should be noted. It is also of interest that a low level of penetration was achieved by most of the hexaploid hybrid accessions examined. This ties in well with the very low in vitro results and probably indicates that pollen that is capable of germination is capable of penetrating the stigma and style.

5.3.4_{iv} Seed-set and seed germination and establishment

This topic is in two parts, the first concerning the production and viability of hybrid seed by Reynoutria species; the second the seed-set and viability of the Reynoutria hybrids. Of the former the best example is that of the production by male-sterile Reynoutria clones of

hybrid seed with F. baldschuanica. This was discovered by accident during an examination of the seed produced by male-sterile Reynoutria plants growing in situations well isolated from known sources of Reynoutria pollen. As part of the work into the role of seed-set in the spread through Britain of Reynoutria taxa it was necessary to collect seed from plants growing in the wild, germinate the seeds, and determine the chromosome number of both them and a rhizome from the parent clones. In all, seed was examined from 19 locations throughout Britain (Table 5.11). It was then something of a surprise to find that the majority of the seed examined was in fact hybrid with F. baldschuanica. The one wild male-fertile population of R. japonica var. compacta (P99) from Scotland examined did actually breed true, as did seed collected from male-fertile R. sachalinensis plants at Amroth (Wales P67,68) and Cirencester (P64). The only known example of inter-specific hybrid Reynoutria seed is that found at Caerynch Hall where a male-fertile R. sachalinensis grows near to a male-sterile octoploid R. japonica (P5), accounting for the 5 hexaploid seedlings in Table 5.11.

In spite of the Reynoutria parents having 44 or 88 chromosomes and a base number of 11, compared with the diploid level and base number of 10 of F. baldschuanica, this hybrid seed is remarkably fertile. Wild-collected seed from Sileby (Leicestershire P25), which had previously been sampled to show its Fallopia parentage, had a germination rate of 76% (Table 5.12) which exceeds the germination rate of the two other octoploid R. japonicas (P179 and P12) in

SEEDLING CHROMOSOME NUMBERS	FEMALE PARENT	R. japonica var. compacta 2n=44		R. sachalinensis 2n=44		R. japonica var. japonica 2n=88	
		No. Seed examined	No. locations	No. seed examined	No. locations	No. Seed examined	No. locations
2n=32				13	3		
2n=44		4	1	13	3		
2n=54						32	11
2n=66				1	1	5	1

TABLE 5.11 CHROMOSOME NUMBERS OF SEEDS AND PARENTAL RHIZOMES COLLECTED FROM WILD BRITISH REYNOUTRIA LOCALITIES

Accession	P No.	wild/ cult.	No. Seeds	Germin ated	% germ.
<u>F. baldschuanica</u>	P152	C	3	1	3
<u>F. baldschuanica</u>	P151	C	8	2	25
<u>F. baldschuanica</u>	P98	C	3	2	66
<u>F. dumetorum</u>	P149	W	26	22	84.6
<u>F. multiflora</u>	P162	BG	14	1	7.1
<u>F. cilinodis</u>	P148	BG	22	3	13.6
<u>F. cilinodis</u>	P156	BG	12	0	0
<u>F. convolvulus</u>	P150d	BG	26	18	69
<u>R. sachalinensis</u>	P57	C	36	26	72
	*P55	C	9	4	44
	*P63	C	30	28	93
	P127	W	30	17	56.6
	P61	C	22	9	41
	P116	C	18	4	22
	*P62	C	17	12	70.6
<u>R. jap. v. compacta</u>	P2b	C	22	20	91
4x <u>R. japonica</u> x <u>R. sachalinensis</u> hybrids	P79a	C	36	31	86
	P125	W	35	19	54
	P126	W	28	15	53.6
	*P78a	C	30	14	46.6
6x <u>R. japonica</u> x <u>R. sachalinensis</u> hybrids	P75c	C	18	12	66.6
	P130	W	15	14	93
	*P32	C	14	5	36
	P31	C	24	16	66
	P17	C	22	19	86
<u>R. japonica</u> 8x	P25	W	25	19	76
	P179	C	10	5	50
	P12	C	35	25	71
<u>R. jap. x R. sach.</u> 8x	*P5Ib	C	4	4	100

* male fertile clone

TABLE 5.12 SEED GERMINATION OF FALLOPIA AND REYNOUTRIA HYBRIDS

the table. These plants were open-pollinated in cultivation and distant from a source of Fallopia pollen, and so were probably inter and intra-specific hybrid seed. On the basis of such small samples I would not like to suggest that inter-generic hybrid seed was more fertile than intra- or inter-specific Reynoutria seed, but it is clearly no less fertile. Another noticeable feature was the speed with which the inter-generic seed germinated - often within 4 or 5 days. Seed produced by artificial hybridization was due to the small numbers involved, routinely germinated by embryo-culture.

I have been able to make a comparison between the seed-set of male-sterile R. japonica clones, both in the presence and absence of Reynoutria pollen. Table 5.13 shows that R. japonica octoploids grown in the Fish tanks had a seed-set of between 2.46 and 6.47% (P12, P179), whilst P183 and P184 growing some 2 miles from the nearest Reynoutria pollen (and where seed had previously been shown to be of intra-generic origin) had a seed-set between 0.15 and 0.23%. Whilst in percentage terms these rather insignificant it must be noted that these plants can produce up to 200,000 flowers per stem and that even 0.15% seed-set produces 283 seeds per stem, and stands containing hundreds of stems are not uncommon. The relatively low percentage seed-sets of the plants grown in the presence of Reynoutria pollen can probably be interpreted as being limited by the resources allocated to seed production rather than any shortage of pollen.

With regard to the production of seed by Reynoutria hybrids,

Taxon	Sex	Chr. No.	Mean No. ochrea per infl.	Mean Ped. per ochrea	Mean Fla. per infl.	No. infls. per stem	Fls per stem	Seed set per stem	1 seed set
<u>R.jap v.comp.</u> P2a	Female	44	57	2.58	147.06	42	6176	516	8.35
<u>R.sach.</u> P55	Male	44	139.5	2.65	369.67	153	56,560	2,780	4.915
<u>R.sach.</u> P115	Female	44	205.5	2.33	478.8	209	100,072	11,323	11.31
<u>N.R.jap. var.</u> <u>comp x R.sach.</u> P13	Male	44	105	3.4	357	70	24,990	141	0.56
<u>A.R.sach. x R.</u> <u>jap.v.comp.</u> P79a	Female	44	*	*	253	74	18,754	6282	33.5
<u>A.R.sach. x</u> <u>R.jap.v.comp.</u> P79c	Male	44	82	6.3	516.6	77	39,778	5	0.013
<u>A.R.jap.v.comp.</u> <u>x R.sach</u> P78a	Male	44	160	3.15	504	104	52,416	357	0.68
<u>A. R.jap. x</u> <u>R. sach.</u> P75b	Male	66	112.5	2.62	294.75	60	17,685	6	0.034
<u>A.R.jap. x</u> <u>R. sach.</u> P75d	Male	66	146.25	2.66	389.03	201	78,195	5	0.0064
<u>A.R.jap. x</u> <u>R.sach</u> P75c	Female	66	122.5	2.66	355.25	335	119,195	790	0.066
<u>A.R.jap. x</u> <u>R.jap. var.</u> <u>comp.</u> P76b	Male	66	92	3.5	322	68	21,896	28	0.13
<u>N.R.jap. x</u> <u>R.sach</u> P31	Female	66	122	2.45	198.9	264	78,909	248	0.31
<u>N.R.jap. x</u> <u>R.sach</u> P32	Male	66	118	2.97	350.5	484	169,623	142	0.084
<u>R. jap.</u> P12	Female	88	182	2.67	485.9	234	113,709	2800	2.46
<u>R. jap.</u> P179	Female	88	130	2.96	384.8	247	95,045	6152	6.47
<u>R.jap.*</u> P183	Female (88)	(88)	146	3.2	467.2	410	191,552	442	0.23
<u>R.jap.*</u> P184	Female (88)	(88)	179.5	3.37	595.94	322	191,892	283	0.15
<u>N.R.jap x</u> <u>R.sach.</u> P51B	Male	88	107	2.85	304.9	242	73,798	607	0.82

* seed collected from plants in the wild.

TABLE 5.13 FLOWER PRODUCTION AND SEED-SET OF REYNOUTRIA

SPECIES AND HYBRIDS GROWING TOGETHER AT LEICESTER.

one has the complication that many of them are male-fertile, some extremely so with scarcely any gynoecium development. These particular plants then have the double disadvantage of less than perfect meiosis combined with the generally lower seed-set encountered when male-sterile and male-fertile plants are compared. Take, for example, R. sachalinensis (Table 5.13), where two plants (one male-sterile the other male-fertile) were grown in identical conditions. The male-sterile clone produced a seed-set of 11% compared to 5% in the male-fertile clone. This difference is amply illustrated by 3 artificial R. japonica x R. sachalinensis hybrids (P75b,c and d), 75b and d being male-fertile and P75c male-sterile (see also Plates 5.22a,b). Seed-set by these male-fertile hexaploids of 5 and 6 seeds per stem, a minute percentage of flower production, compares dramatically with the male-sterile clone with 790 seed per stem. The same trend is repeated with two artificial hybrids at the tetraploid level (P79a,c), the male-sterile plant producing over 6000 seeds compared with only 5 for the male-fertile clone. This is obviously a reflection of the greater fertility of the tetraploid Reynoutria hybrids, the comparably low figures for male-fertile clones presumably being accounted for by the poor development of the gynoecium in these plants. With P79c, 5 seeds from 39,000 flowers amply illustrates the impracticability of trying to follow changes in sex-expression within these particular clones. The octoploid hermaphrodite hybrid P51b produced over 600 seeds per stem which, although easily the highest seed production for a male-fertile hybrid, was only 25% of the total seed of male-fertile R. sachalinensis (P55). The

chromosome numbers of seed-set by Reynoutria hybrids both in the wild and in cultivation in Leicester is shown in Table 5.14. Tetraploids and the octoploid hybrids seem all able to produce euploid offspring, but not so the hexaploid clones. The hexaploid R. japonica x R. sachalinensis clones produced aneuploid plants with chromosome numbers between 48 and 77, (euploids are 55 and 77). The chromosome numbers in the seventies are explained by the fact that the plant (P52) which gave rise to them grows next to the octoploid hermaphrodite at Dolgellau. It should be noted that these aneuploid counts are the only ones that I have ever come across in Reynoutria. Further, although the numbers 55 and 77 are numerically euploid at the pentaploid and heptaploid level, the existence of plants with more or less chromosomes than these suggests that they are not necessarily genetically euploid and may well be deficient and duplicate with respect to certain chromosomes.

As mentioned earlier, the establishment from seed of Reynoutria hybrids in this country is still very much at the anecdotal stage of knowledge. Hybrid seedlings have been observed in the fish tanks at Leicester, but were not observed in their second season: seed of R. japonica x F. baldschuanica constitution was overwintered outdoors on top of the Adrian building at Leicester in the winter of 1985/6 and was observed to germinate naturally and freely in April and May 1986. The R. japonica x F. baldschuanica hybrid found growing in old Railway sidings in London must rank as the only known example of recruitment in the wild of Reynoutria plants from seed.

Female Parent	Accession/location	Wild or Cultivated	Chromosome numbers of seedlings
<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u> 2n=44	Gomshall P33 Cheshunt P125,6 Circencester P131	Wild Wild Wild	2 x 2n=32 5 x 2n=32 2 x 2n=44
<u>R. japonica</u> x <u>R. sachalinensis</u> 2n=66	Maam P31 Circencester P132 Circencester P130 Dolgellau P49	Cult. Wild Wild Wild	2n=48(1),55(1),57(1) 1 x 2n= Ca 53 1 x 2n=49 2n=C77(1),76(1),75(1)
<u>R. japonica</u> x <u>R. sachalinensis</u> 2n=88	Dolgellau P51b	Wild	2 x 2n=88

TABLE 5.14 CHROMOSOME NUMBERS OF THE SEEDLINGS OF HYBRID REYNOUTRIA PLANTS.

Two different batches of seed germination have been tried. The first, shown in Table 5.12, is an attempt to get the maximum germination in carefully controlled conditions, including the removal of the pericarp with concentrated H_2SO_4 following the technique of Justice (1941). The other results (Table 5.15) reflect more the conditions the seeds might face in the wild and are hopefully a measure of what one could expect to germinate. Table 5.15 indicates that reasonable levels of germination might be expected of seed produced by hybrid plants, the relatively poor germination rates of seed from male-fertile plants being a point to note. Some extremely high germination rates (up to 100%) are recorded in Table 5.12. Of particular interest in this table are the germination percentages of wild-collected Cheshunt R. sachalinensis (56.6%), Cheshunt hybrids a and b (54 and 53.6%), and octoploid R. japonicas P125 and P179 (76% and 50%), since in all these cases the male parent was F. baldschuanica!

From these results there seems to be no reason why these seeds should not germinate perfectly well in the wild. surviving the first winter is probably the problem. This apart, there are in fact no reports of Reynoutria seedlings in the wild in spite of the repeated attempts of various people to find them.

Accession	Wild or Cult.	Ploidy Level	% Germ.	
<u>R. jap.v.comp.x R. sach.</u> (art. hybrid) P78a	C	4x	20	
Put. <u>R. jap.x R. sach.</u> P30	C	6x	15	
Put. <u>R. jap.x R. sach.</u> P29	C	6x	0	
Put. <u>R. jap.x R. sach.</u> P32	C	6x	0	
Put. <u>R. jap.x R. sach.</u> P31	C	6x	37	
Put. <u>R. jap.x R. sach.</u> P31	C	6x	38	
Put. <u>R. jap.x R. sach.</u> P52	W	6x	0	
Put. <u>R. jap.x R. sach.</u> P129	W	6x	6	
Put. <u>R. jap.x R. sach.</u> P130	W	6x	28	
Put. <u>R. jap.x R. sach.</u> P51	W	8x	0	

TABLE 5.15 SEED VIABILITY OF R. JAPONICA X R. SACHALINENSIS
HYBRIDS

	Treatment	No. Seeds	Period observed	Sex
	20°C light	10	3 weeks	male-fertile
	15°C light	13	3 weeks	male-fertile
	15°C light	7	4 weeks	male-fertile
	25°C light	4	4 weeks	male-fertile
	25°C light	19	3 weeks	male-sterile
	20°C light	13	3 weeks	male-sterile
	25°C light	12	8 days	male-sterile
	20°C light	17	3 weeks	male-sterile
	20°C light	25	3 weeks	male-sterile
	25°C light	8	8 days	hermaphrodite

5.4 DISCUSSION

5.4.1 HYBRIDIZATION

The first point which I wish to emphasize is the vital role played by cytology in this investigation. The production of reliable chromosome number determinations at Leicester had a profound effect on our understanding of the Reynoutria taxa in the British Isles. Not only were hexaploid plants found, but also these plants differed morphologically from other R. japonica and R. sachalinensis plants examined. The discovery of plants intermediate in both morphology and chromosome number - in other words hybrids - led to a sharpening of perceptions as to what precisely constituted the parental taxa. Once the species boundaries had been drawn it then became clear that in R. japonica, what had been taken as merely a paucity of male plants, was in fact, a complete absence of male-fertiles, as existing 'males' were all found to be hybrids. Since the male-sterile octoploid was by far the most common Reynoutria, these findings had an important bearing on notions of how these plants reproduced and spread in the British Isles.

This study has resulted in the discovery of 2 different inter-specific Reynoutria hybrids, one of which (R. japonica var. compacta x R. sachalinensis) is previously unrecorded. More interestingly it has been found that seed taken from male-sterile Reynoutria plants in the wild is always the result of hybridization and usually with the frequently grown garden plant Fallopia baldschuanica or Russian Vine.

This inter-generic hybrid is made all the more remarkable by the disparity in both ploidy level (8x versus 2x) and base number (11 versus 10), by the ease with which it can be artificially synthesised, and by the very high germinability of the seed. It is then perhaps fortunate for the inhabitants of this small island that these hybrids exhibit negative heterosis for the havoc that 'mile a minute giant knotweed' could wreak on the environment is unthinkable! Stace (1975) gave two instances, Poa annua x P. infirma and Anthemis arvensis x Tripleurospermum inodurum of viable hybrid seed being collected from seed heads in the wild, but where the F₁ hybrid has not itself been recorded. There are many different causes for this phenomenon, and until 1987 Reynoutria x Fallopia was an addition to this list. However, in that year, as a result of my having previously exhibited cultivated plants of this combination at a BSBI meeting, D. Bevan found a R. japonica x F. baldschuanica hybrid growing in some disused railway sidings in Haringey Middlesex, (this area has since been designated a nature reserve). The plant was a large one and had obviously been established for many years, possibly since soon after the railway sidings had been abandoned. As this hybrid has not been found growing in the wild anywhere since then it would be well to consider what special conditions enabled it to get established there and nowhere else. Whilst I have demonstrated that seeds of this combination will happily overwinter outdoors in potting compost and germinate in the spring, and that plants grown in the greenhouse are perfectly hardy if planted out in their 2nd or 3rd year, I have no information about first-year survival of seedlings

outdoors. Since there are no records of Reynoutria establishment in the wild it is strange that a hybrid between a Reynoutria and Fallopia should be the one to survive. This is particularly true when one considers that such a plant is also a hybrid in terms of life-form, for R. japonica is herbaceous, overwintering with rhizomes, and F. baldschuanica is a deciduous woody plant without rhizomes. A hybrid between these two growth forms would have special difficulties with the onset of winter, since the parents have competing overwintering strategies. Two factors about this site recommend it for the establishment of such a hybrid. First, being in London, there is a chance of escaping a serious frost in the first winter; and second, a recently abandoned railway goods-yard might be expected (especially as it was granite-setts) to be initially free of competing ground-cover.

Containing 4 Reynoutria genomes to 1 Fallopia genome, the pentaploid hybrid is very similar to R. japonica in form and habit, and it is possible that it follows the Reynoutria mode of overwintering in its first season (suberitized rhizome and two underground buds). This is unlikely to be the case with the triploid $2n=32$ hybrids which are formed with R. sachalinensis or R. japonica var. compacta and F. baldschuanica. The 2:1 Reynoutria:Fallopia genome ratio is probably responsible for the much greater phenotypic resemblance to F. baldschuanica than to the Reynoutria parent. This is much more intermediate than the pentaploid, bearing stems that become woody and overwinter in part, and short rhizomes which lack the horizontal spread and extent

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of the Reynoutria parent.

The results of the chromosome counts of seed collected from wild Reynoutria clones has important implications regarding the spread by seed of these plants. At only five of the twenty-six localities where seed was collected did the seedlings have the same chromosome number as the parent (see Tables 5.11 and 5.14). Further, two of these were hybrids at the 4x and 8x levels and the other three were male-fertile R. sachalinensis plants. On no occasion was 'true' seed taken from either male-sterile R. japonica var. japonica or R. sachalinensis - the most commonly found taxa in this country. All seed taken from these male-sterile plants was of hybrid parentage, either with F. baldschuanica (14 localities) or R. sachalinensis (one locality). The seed produced by most of the hybrids was again either hybrid with F. baldschuanica or aneuploid and lacking vigour. The absence of established aneuploid Reynoutria plants in the wild suggests little or no recruitment of Reynoutria plants from hybrid seed.

The causes of all this hybridization are two-fold. firstly, plants of R. japonica var. japonica introduced to the British Isles all seem to belong to the same male-sterile clone; and secondly, the apparent absence of breeding-barriers both within Reynoutria and between Reynoutria and F. baldschuanica. A more minor role is played by insect pollinators.

The spread through Britain of a male-sterile clone of R.

japonica presented opportunities for hybridization that would not have occurred in its native localities, since, even if sympatric with related taxa, it is doubtful whether (due to certation effects) a small quantity of haploid pollen could have competed effectively on the Reynoutria style in the presence of vast amounts of tetraploid pollen. The apparent complete lack of breeding barriers between F. baldschuanica and male-sterile R. japonica and R. sachalinensis plants would suggest that they are not sympatric in their countries of origin.

The native distribution in China and western Asia of F. baldschuanica and R. japonica is shown in Figures 2.4 and 2.5. F. baldschuanica has a north-westerly distribution, whilst that of R. japonica is south-easterly, and there is some overlap in the Chinese provinces of Szechwan, Shensi and Honan. Some of this could be more apparent than real, since my practice of putting incompletely localized reports in the centre of the province might mask a northerly distribution in F. baldschuanica and a southerly one for R. japonica. In any case, even if they are geographically sympatric I suspect that ecologically they are not, F. baldschuanica preferring the higher drier rocky terrain. Since R. japonica var. compacta and R. sachalinensis do not occur in China, and F. baldschuanica is not sympatric with R. sachalinensis in Russia and is not found in Japan there is reason to suspect that breeding barriers between these taxa would not evolve.

Comparison of Figures 2.3, 2.4 and 2.5 shows that F.

multiflora is geographically sympatric with both F. baldschuanica and R. japonica, although I do not have enough information to say whether or not they are ecologically sympatric. The likelihood (at least in the case of R. japonica and F. multiflora) that ecological sympatry occurs would, assuming that the flowering period and pollinators overlap, be more likely to have led to the evolution of breeding barriers. It would, though, be extremely difficult to identify a hybrid between an octoploid R. japonica and F. multiflora without access to the chromosome number.

Whilst there are certainly year to year fluctuations in the amount of seed set by British Reynoutria plants (the summer of 1987 being particularly good, that of 1986 very poor) I suspect that pollinator behaviour also has an effect. Some of the Welsh R. japonica sites from which hybrid seed has been gathered are at least a mile or two from the nearest source of F. baldschuanica, and pollen transfer over this sort of distance requires dedicated pollinators. Until a few years ago I had not seen Reynoutria plants visited by bees; bee-mimics house-flies and other dipterans were the most common visitors. Whilst such species are undoubtedly effective at pollination in Japan, where the male-fertile and male-sterile plants are more evenly distributed, it is unlikely that they could transfer pollen for a mile or more. As stated in Chapter 4 bees and wasps are known to be important pollinators in Japan, and I consider it possible that British bees have been somewhat conservative in their acceptance of the alien R. japonica and F. baldschuanica as appropriate sources of nectar. At any rate, they are

now often observed on Reynoutria and Fallopia plants.

Another point worth noting is the unidirectional nature of the Reynoutria/Fallopia hybridization; this is of course due to the fact that the Reynoutria plants concerned are male-sterile and that F. baldschuanica is an hermaphrodite. This is not quite the whole story, for it is not impossible for R. sachalinensis pollen to be transferred to an F. baldschuanica stigma. Apart from the question of competition from its own pollen, the stigma of the F. baldschuanica plants examined seems to be a remarkably unreceptive structure, and I have been unable to demonstrate convincing penetration of the style by its own or any other pollen (Tables 5.1 and 5.9), nor have I been able to produce any hybrid seed with F. baldschuanica as female parent. The existence of a self-incompatibility reaction is responsible for the failure of selfed pollen, but other factors may be responsible for the failure to produce the Reynoutria x Fallopia hybrids reciprocally. First of these is the possibility that the F. baldschuanica (generally propagated by cuttings) commonly grown in Britain is infertile. There are several reports of its poor seed-set, and the plant in my own garden produces a very small quantity of fertile seed. In crosses between the ploidy levels it has been found (Watkins, 1932) that, when the pollen:style tissue ratio is lowered from the normal 1:2 to 2:2 in a tetraploid male times a diploid female, the crosses usually fail. The reciprocal cross of diploid male times tetraploid female raises the ratio to 1:4 and is more often successful. In the case of a diploid male times an octoploid female this

ratio is 1:8, and 4:2 in the reciprocal cross. This has been demonstrated in several genera (Stace 1975) and fits well the evidence available here. However, it should be noted that it is not universal; in Prunus for example interploidy crosses succeed best if the diploid is the female.

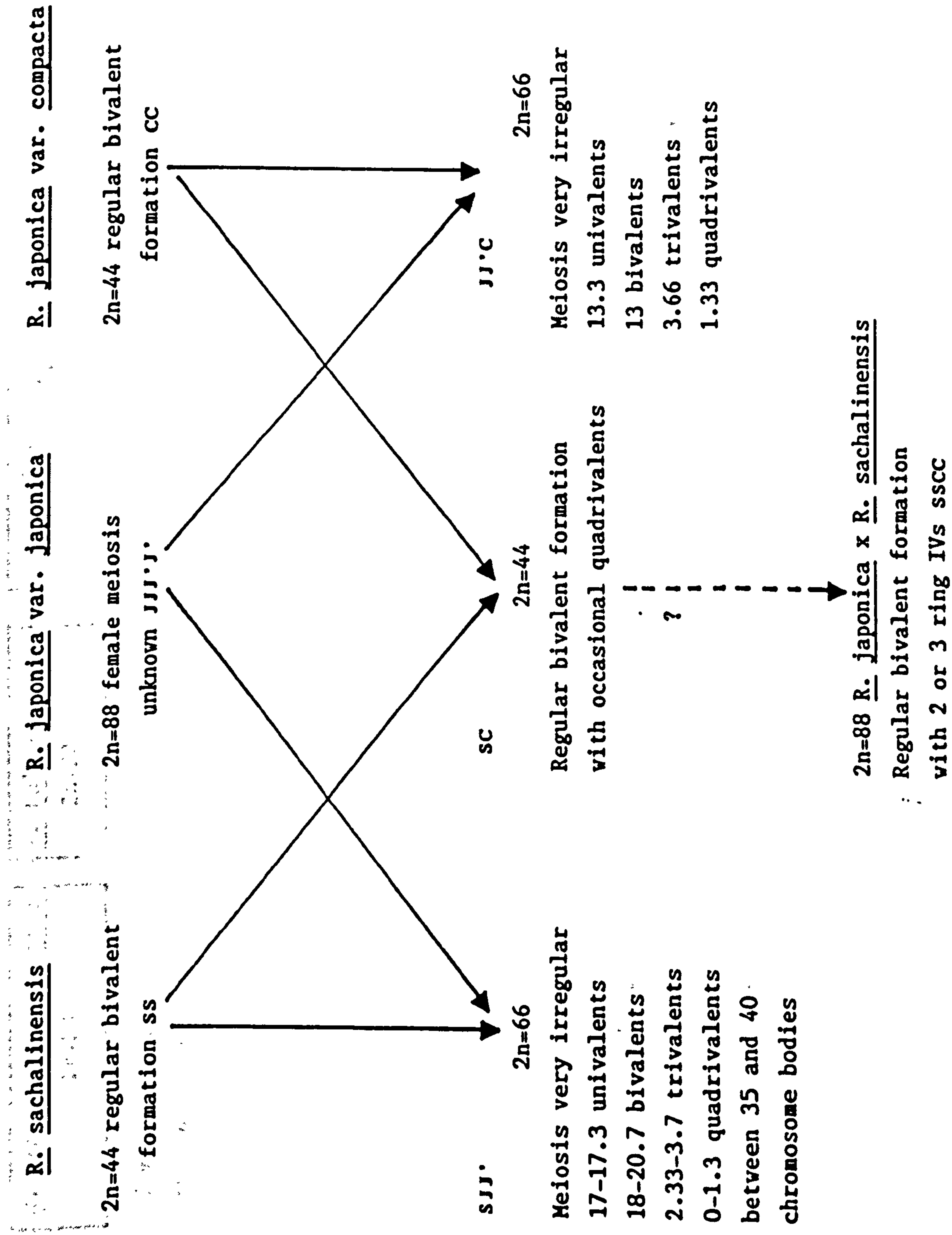
5.4.2 CYTOLOGY

Summaries of the hybrid combinations and of the parental and hybrid meiotic behaviour are given in Figures 5.10 and 5.11. Starting with the male-sterile octoploid R. japonica it will be noted that, although I have not studied female meiosis in this species, I have been able to examine the male meiosis of an octoploid R. japonica x R. sachalinensis cross. This clearly shows that regular meiosis is possible at the octoploid level and if a hybrid can produce regular meiosis the octoploid species would be expected to do no worse. The regular meiotic segregation of the R. japonica var. japonica is also vouched for indirectly by the examination of seed produced by it, which has in all cases been exactly euploid. The meiotic behaviour of the pentaploid R. japonica x F. baldschuanica hybrid is not only most clear-cut, but holds the key to what is occurring amongst these hybrids. Meiosis in these plants regularly gives 22 bivalents and 10 univalents, which I interpret as pairing within the tetra-haploid Reynoutria genome, leaving the 10 Fallopia chromosomes unpaired (Plate 5.9). This is an extremely interesting result since it is an example of good autosyndetic pairing and is indicative of the existence of

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Although the assumptions in the text and the arrangement of the karyotypes (Figs 3.6- 3.9 & 5.1-2) of the polyploid Reynoutrias implies an autopolyploid origin, this is not the only interpretation, since they could be of allopolyploid ancestry. In Figs 5.10 and 5.11 a degree of diploidisation is recognised and letters are allocated on the basis of one per diploid genome set in the polyploid Reynoutria taxa.

FIGURE 5.10 MEIOTIC BEHAVIOUR AND GENOMIC FORMULAE FOR INTER- AND INTRA-SPECIFIC REYNOUTRIA HYBRIDS



* See overleaf

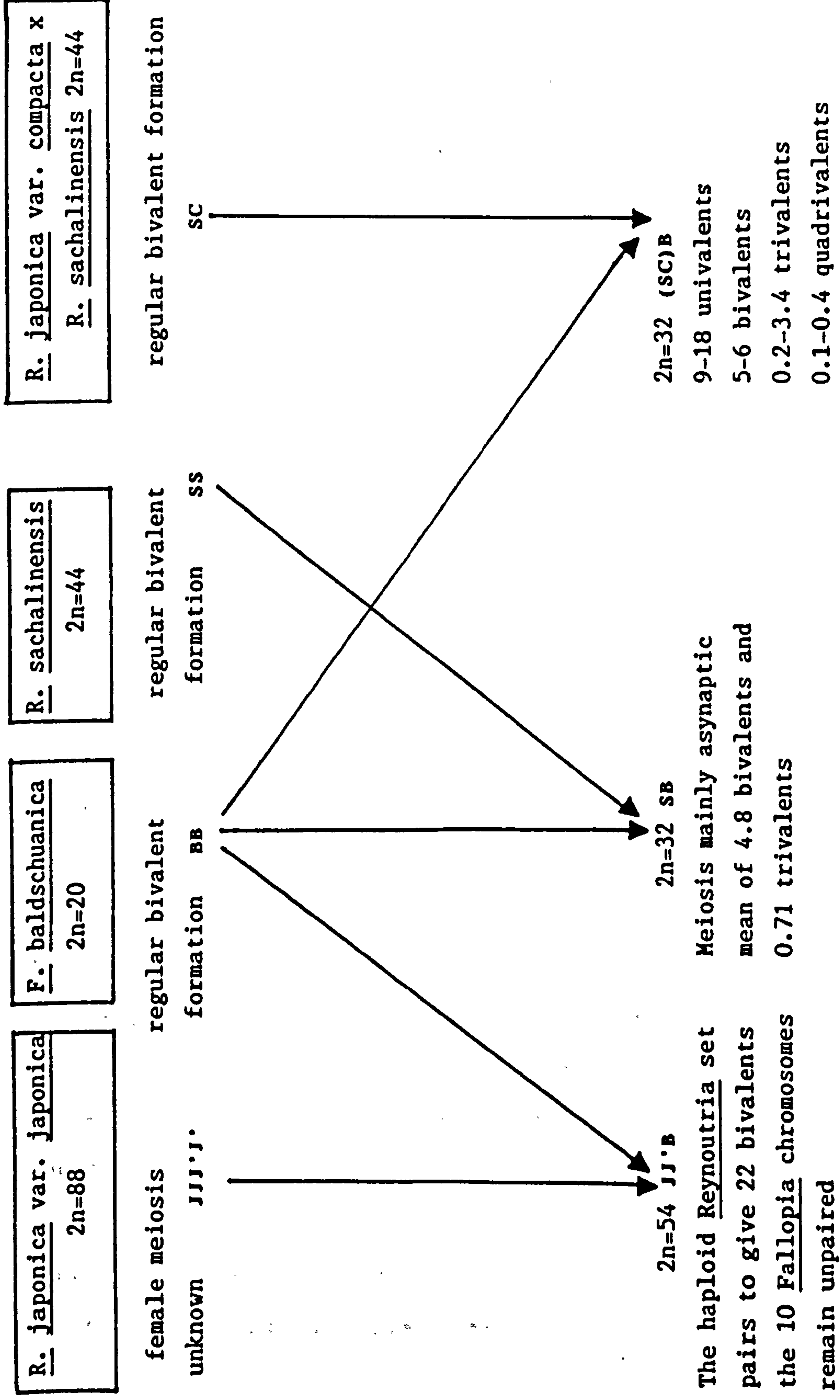


FIGURE 5.11 MEIOTIC BEHAVIOUR AND GENOMIC FORMULAE FOR INTER-GENERIC HYBRIDS

some method of multivalent suppression in the 8x parent. Observation of the meiotic behaviour of the di-haploid sets of R. sachalinensis and R. japonica var. compacta x R. sachalinensis in their hybrids with F. baldschuanica shows that in the case of R. sachalinensis hybrids very little pairing occurs (Figure 5.11). In contrast the meiosis of some plants of (R. japonica var. compacta x R. sachalinensis) x F. baldschuanica reveals as many as 10 bivalent equivalents, including up to 3.8 multivalents. These multivalents suggest that some degree of homology still exists between the Reynoutria and Fallopia genomes. It must be borne in mind that these figures were rather difficult to interpret, and the possibility of some error should not be ignored.

Turning then to the inter- and intra-specific Reynoutria hybrids, regular meiosis (apart from the occasional ring multivalent) is found in R. japonica var. compacta x R. sachalinensis and in the octoploid R. japonica x R. sachalinensis hybrids. The position is quite different with the hexaploid hybrids formed by crosses between octoploid R. japonica and the tetraploid Reynoutria taxa. In these, meiosis is most irregular, although, particularly in the R. japonica var. japonica x R. sachalinensis and to a lesser extent in R. japonica var. japonica x R. japonica var. compacta, there appears to be a core of about 22 bivalents and multivalents.

Whilst the haploid genome of R. japonica var. japonica is capable of forming bivalents, that of R. sachalinensis is

not. This suggests that the tetraploid is considerably more ancient than the octoploid and that in the course of time various mutations and inversions have occurred effectively diploidizing the plant so that the base number has become 22 in terms of homology. The octoploid on the other hand has probably not been around long enough for full diploidization to occur since good pairing can occur in the haploid chromosome set. On the basis of this one can assign letters to the different genomes ($x=22$ in Reynoutria and 10 in Fallopia). Octoploid R. japonica has the genomic formula JJJJ, R. japonica var. compacta CC, R. sachalinensis SS and F. baldschuanica BB. Viewed from this angle the fertility of the R. japonica var. compacta hybrid results from the homology between the S and C genomes, whilst the infertility of the hexaploids is caused by there being an unpaired S or C in the presence of two J genomes, explaining the more or less 22 bivalents plus multivalents found. Thus, in the triploid hybrids, the one involving R. sachalinensis has a genomic formula of SB. With two unpaired genomes one would not expect a great deal of pairing, since it would have to be either amongst the chromosomes of the diploidized S complement or between Fallopia and Reynoutria chromosomes. the position in the double hybrid (R. japonica var. compacta x R. sachalinensis) x F. baldschuanica is a little more complex, since if the assumption that the S genome is pairing with the C genome is correct, any offspring in this hybrid with F. baldschuanica would, due to random segregation of bivalents be a random mixture of chromosomes from the S and C genomes. The presence of the occasional quadrivalent in the SC parent suggests that there is some

homology between the S and C genomes and this might explain the greater degree of pairing found in some plants of this combination.

In assigning my species and hybrids a genomic formula I have deliberately chosen letters based on the specific names rather than ones which imply degrees of genomic homology. Classical techniques of the genome analysis of species and hybrids seek to assign genome homologies and auto- or allopolyploid origin to polyploids. Such techniques fail when confronted with multivalent suppressors, which are clearly indicated in the case of R. japonica var. japonica. This is because a multivalent suppressor can disguise the meiotic evidence of autopolyploidy or segmental allopolyploidy on the one hand, and on the other give false evidence of genomic homology between taxa in the case of crosses between diploids and tetraploids. It is best to avoid making such conclusions when dealing with taxa that possess these multivalent suppressors.

5.4.3 EVOLUTION

What relevance if any do these new hybrids have to the British flora both in terms of long-term survival and in the generation of further novel variation and new species? Taking the interspecific Reynoutria hybrids first, we have already seen that hybrids between R. japonica var. compacta and R. sachalinensis ($2n=44$) have regular meiosis and good pollen fertility. In terms of seed-set one male-sterile hybrid of this constitution (P79a; Plate 4.3b and Table

5.13) achieved a seed-set of 33% in cultivation, a result which betters any Reynoutria accession under observation. In view of the fact that male-sterile plants are obligate out-breeders it may be assumed that much of this seed will have resulted from back-crossing to one or other of the parental taxa. It is equally possible that the male-fertile plants of this combination back-cross to the male-sterile parental taxa, the existence of self-incompatibility making self-pollination unlikely. We have therefore a situation where back-crossing by the male-fertile hybrid leads to gene-flow between the two species in both directions, whilst that involving male-sterile hybrids involves gene-flow in only one direction (japonica to sachalinensis).

The hexaploid Reynoutria hybrids have irregular meiosis and much reduced pollen fertility, and seed that has been cytologically examined has been found to be generally aneuploid. Whilst the R. japonica x R. sachalinensis plants are large and vigorous, R. japonica var. japonica x R. japonica var. compacta hybrids are rather weak and straggling and would not fare very well in the wild. I would not expect the aneuploid seed from hexaploid hybrids to stand much chance of getting established, but it is not unusual for the occasional balanced diploid or tetraploid gamete to be produced by chance from the irregular meiosis of a sterile hexaploid. When such balanced gametes lead to the production of a plant, by back-crossing, it holds out the opportunity for the restoration of fertility and the possibility of gene flow between the species as Stace and Ainscough (1984) demonstrated in the case of a sterile

Festulopia hybrid. In terms of long-term survival and propagation a vigorous rhizomatous Reynoutria plant has no real necessity for fertility or recruitment from seed as the spread in Britain of the male-sterile octoploid amply illustrates. In the British Isles the potential for intr-gression and gene flow is much greater in the male-fertile clones than in the male-sterile ones, since a male-sterile hybrid is unlikely to be anywhere near Reynoutria pollen, whereas a male-fertile one is unlikely to be far from a male-sterile R. japonica. Male-sterile Reynoutria hybrids do of course have the potential of hybridizing with F. baldschuanica and that has occurred at Cheshunt and Gomshall where R. japonica var. compacta x R. sachalinensis plants have given rise to the triploid hybrid with F. baldschuanica.

In terms of generation of new variation it is to the intergeneric Reynoutria x Fallopia hybrids we must look. In contrast to the interspecific Reynoutria hybrids, where variation is restricted to rather trifling differences in leaf shape, plant size and chromosome number, and in any permutation one is still dealing with a recognisable Reynoutria plant, these inter-generic hybrids are quite new in terms of growth form and gross morphological variation. The inter-generic hybrids found so far are triploid ($2n=32$) or pentaploid ($2n=54$), though I have not entirely given up hope of finding a tetraploid ($2n=43$) in seed from a hexaploid Reynoutria. Whilst of course triploids and pentaploids are completely sterile in terms of equal and balanced meiotic segregation, they are by no means incapable

of reproducing. In the genus Taraxacum, for example, triploid and pentaploid (among others) taxa occur, and although they generally avoid sterility by agamospermy, some retain meiosis and triploid and tetraploid offspring can result from a triploid plant due to fertilization of unreduced gametes and a suppression of precocious embryony (Richards 1973). Sexually reproducing pentaploids are found in the genus Rosa, (Gustaffsson and Hakansson 1942) where in the R. canina group only two of the five sets pair at meiosis. In the male meiosis it is one set of 7 chromosomes that goes into the pollen grain, whilst in the female these 7 are joined by three unpaired sets to give tetraploid female gametes, the pentaploid number being regained at fertilization. Stace and Ainscough (1984) found that in a sterile pentaploid Festulopia hybrid a chance back crossing with a hexaploid F. rubra led to the restoration of fertility in a hexaploid seedling (due to the presumed production of a balanced triploid gamete by the pentaploid). Thus it can be seen that sterility and rapid elimination is not the inevitable fate of triploid and pentaploid hybrids. Another escape from sterility is by amphiploidy. Generally the more sterile the F_1 hybrid the more fertile the amphiploid, since the less the pairing in the F_1 the fewer the quadrivalents in the doubled up plant.

It seems that in all these hybrids the main barrier to their success lies in their initial establishment from seed in the wild. The absence of reports of Reynoutria hybrids establishing themselves from seed in the British Isles need not mean that it does not occur, rather that it is of

infrequent occurrence and perhaps limited to the warmer southern parts of the country when a warm summer and autumn are followed by a mild winter. That a pentaploid R. japonica x F. baldschuanica was able to get established from seed in London ought to imply that the much more vigorous R. japonica x R. sachalinensis hybrids should stand a corresponding greater chance of survival. Once these triploid and pentaploid Reynoutria x Fallopia hybrids become established the opportunity then exists for the production of new species. As we have seen the pentaploid hybrid produces 22 bivalents and 10 univalents, the laws of probability dictate that every so often these will segregate to give a cell with 22 Reynoutria and 10 Fallopia chromosomes. Should the latter lead to the formation of an egg nucleus that is subsequently fertilized by a F. baldschuanica male gamete there is a good chance fertility will be restored in this new tetraploid, and a new amphiploid species will have been produced albeit by a rather circuitous route.

On the other hand meiosis in the triploid hybrids is often so irregular that the first division fails leading to formation of restitution nuclei and unreduced pollen grains or egg cells (Plates 5.5, 5.6 and 5.22). Fertilization of such an egg would again lead to the production of an amphiploid with improved fertility - fertility being dependent on the degree of pairing obtained by the haploid Reynoutria genome.

Once again, Man's interference with the environment in terms

of bringing previously non-sympatric genera together, in an unbalanced population as far as sex-expression is concerned and in areas where open habitats (waste ground) are readily available, has led to the production of some interesting inter-generic hybrids with the possibility of new species production.

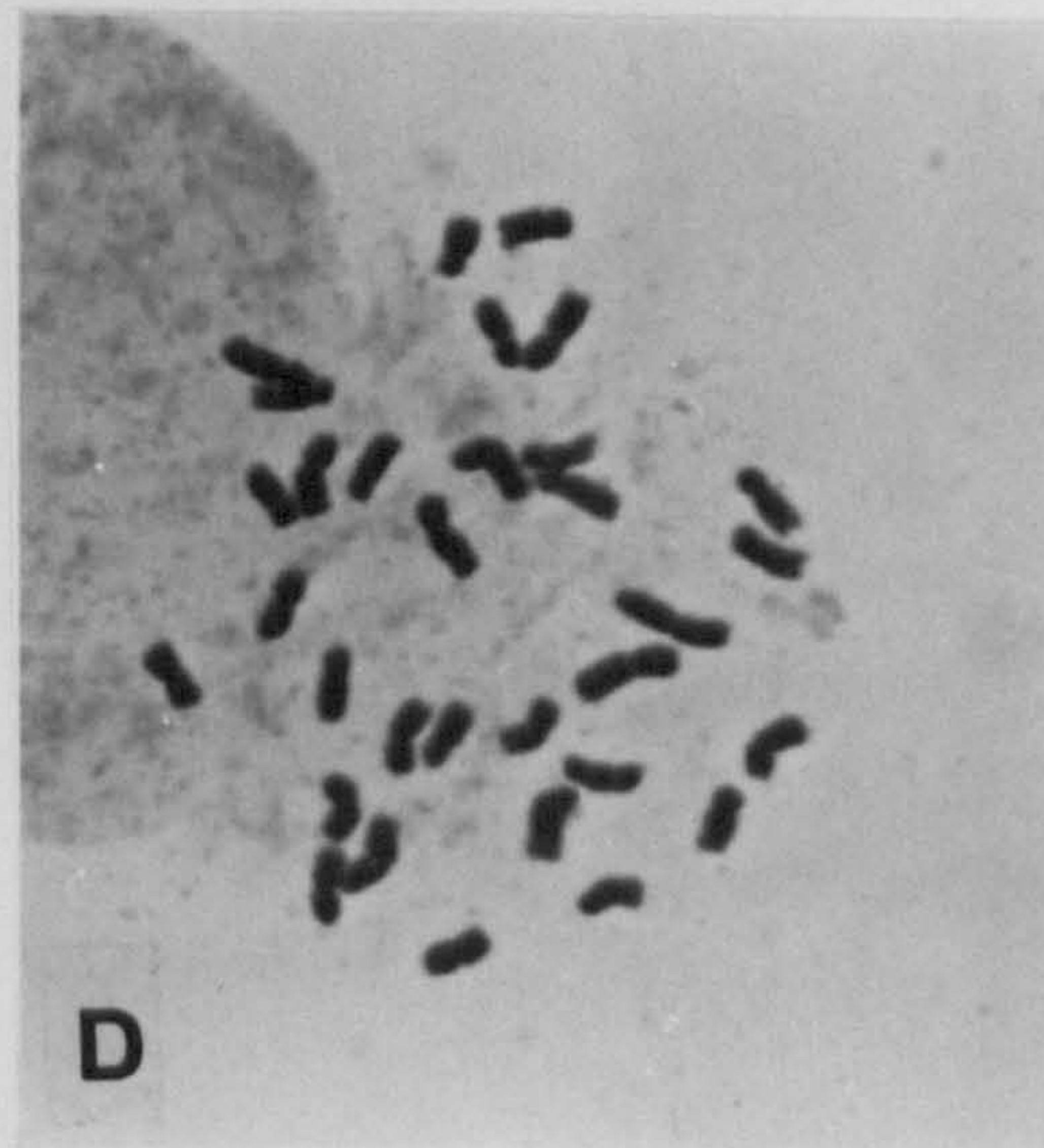
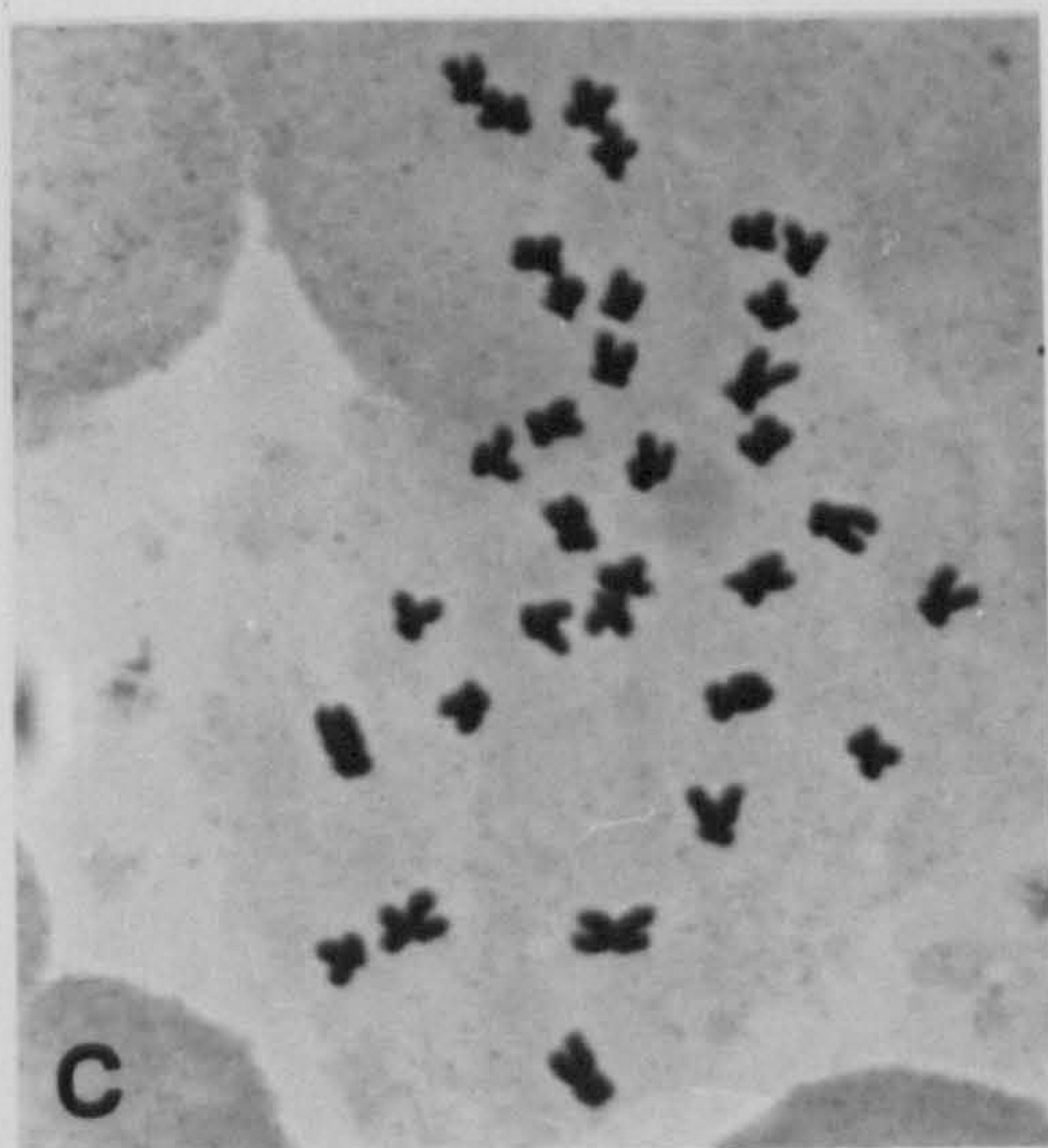
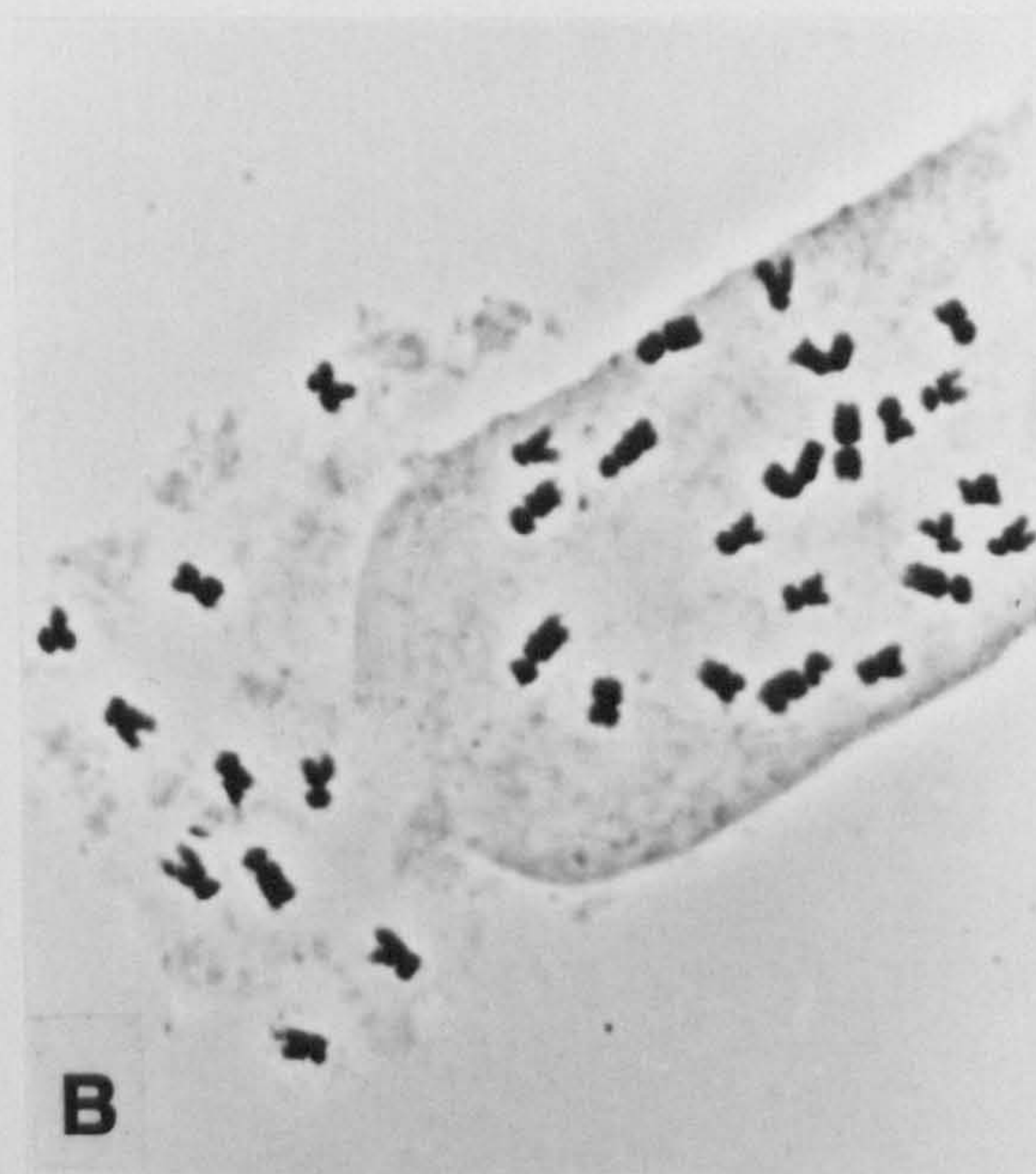
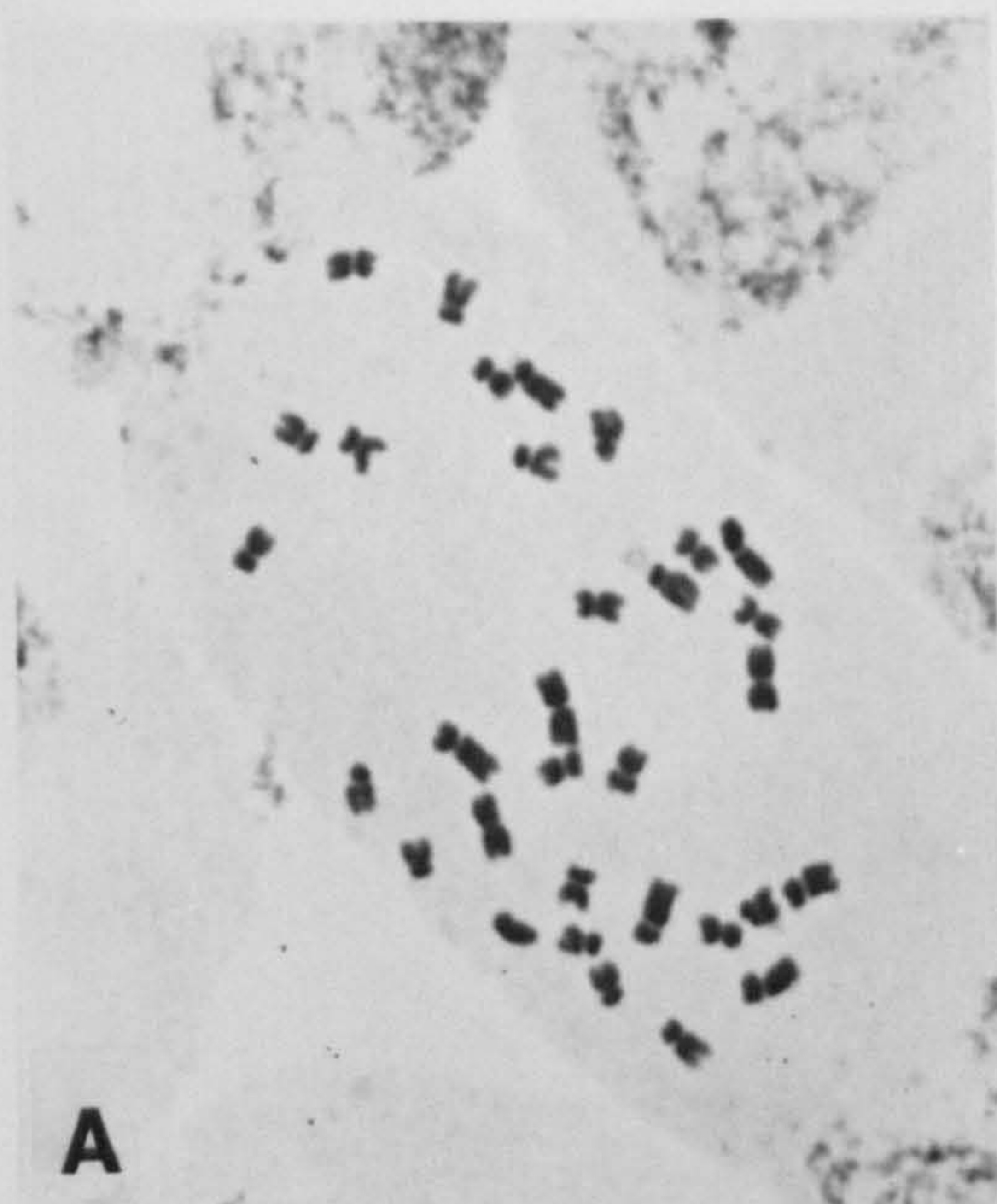


Plate 5.1 Triploid Mitosis $2n=32$

A, B artificial *R. sachalinensis* x *F. baldschuanica* hybrids, P101b,a
Note 2 satellites in A

C Putative 4x *Reynoutria* x *F. baldschuanica* hybrid P146c

D *R. japonica* var. *compacta* x *F. baldschuanica*

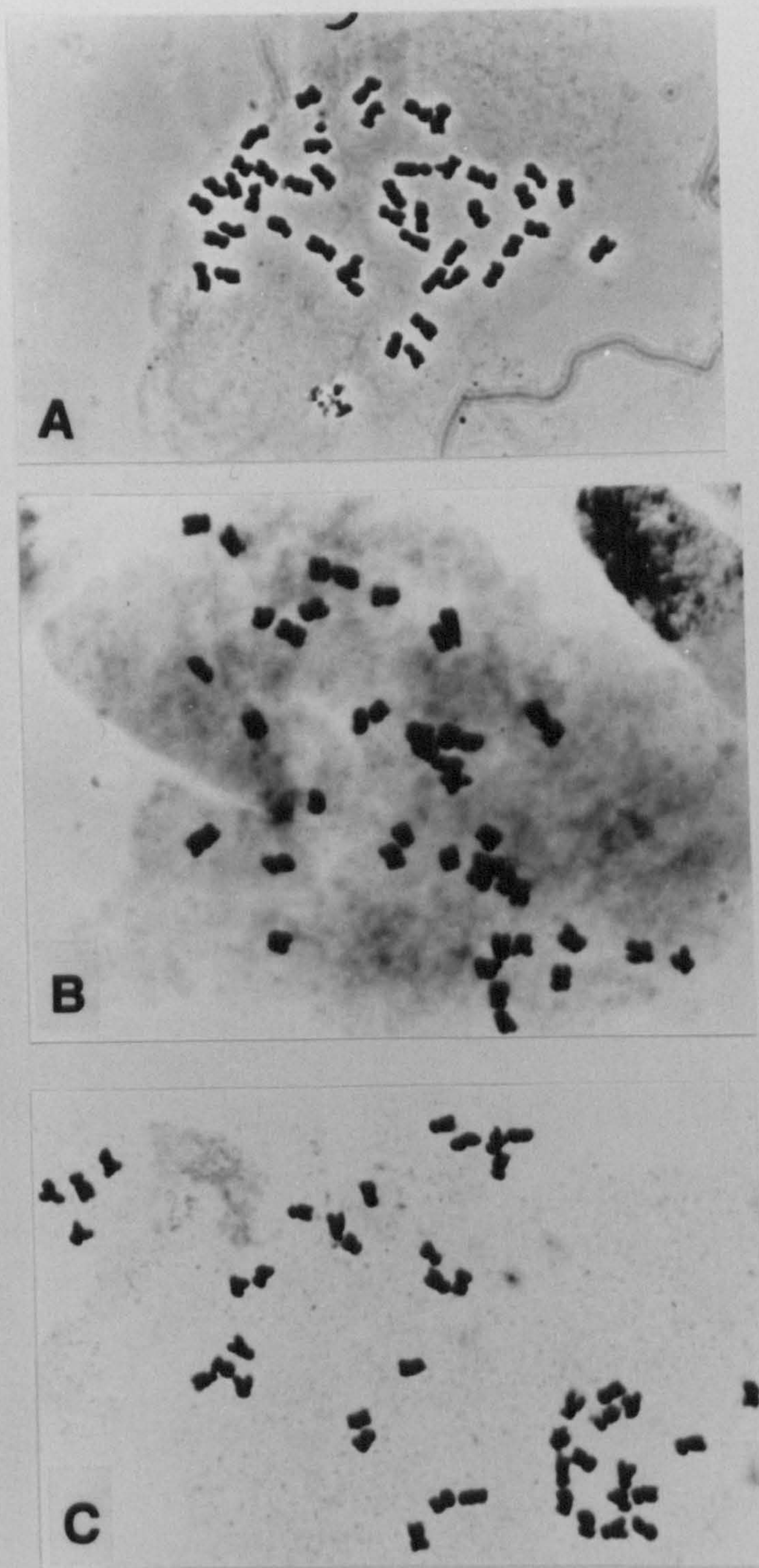


Plate 5.2 Tetraploid mitosis

- A** Artificial *R. sachalinensis* x *R. japonica* var. *compacta* P79c
2n = 44
- B** Artificial *R. sachalinensis* x *R. japonica* var. *compacta* P79b
2n = 44
- C** Putative *R. japonica* x *R. sachalinensis* hybrid P33

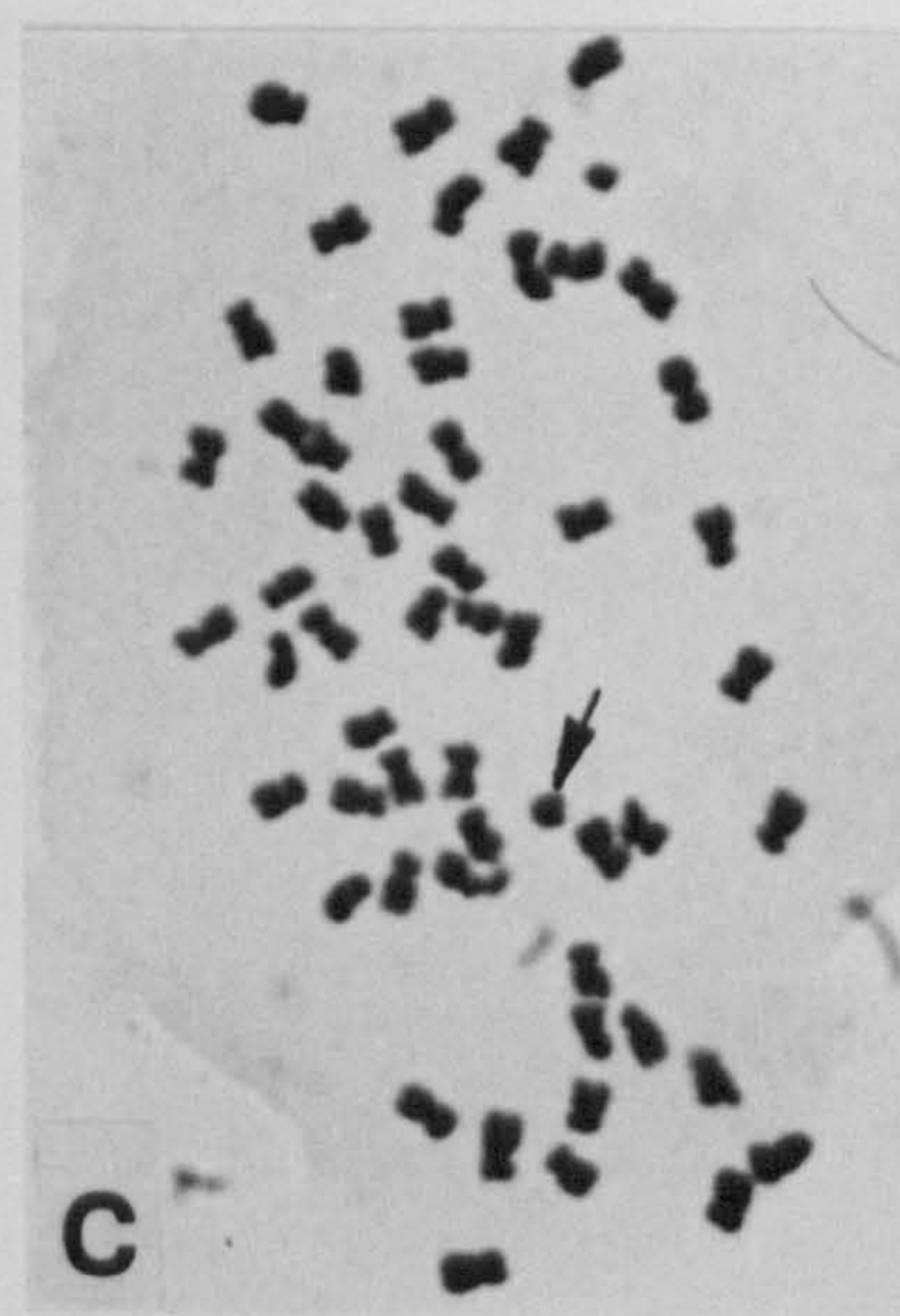
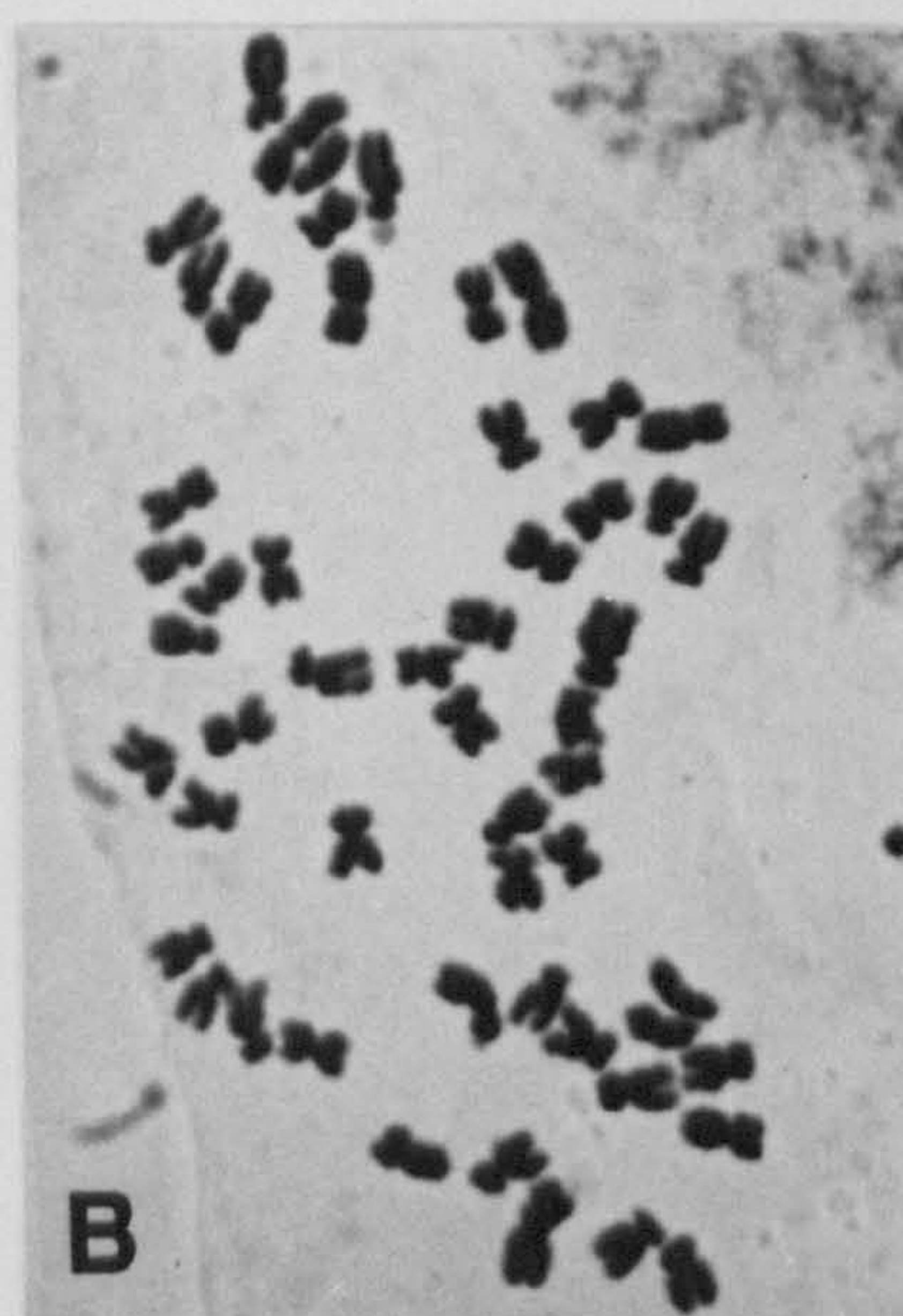


Plate 5.3 Pentaploid Mitosis

- A** *R. japonica* x *F. baldschuanica* artificial hybrid P94a $2n = 54$
- B** *R. japonica* x *F. baldschuanica* natural hybrid (from seed) P91b $2n = 54$
- C** Seed from a hexaploid female (P31) P85b $2n = 55$ + telocentric fragment arrowed

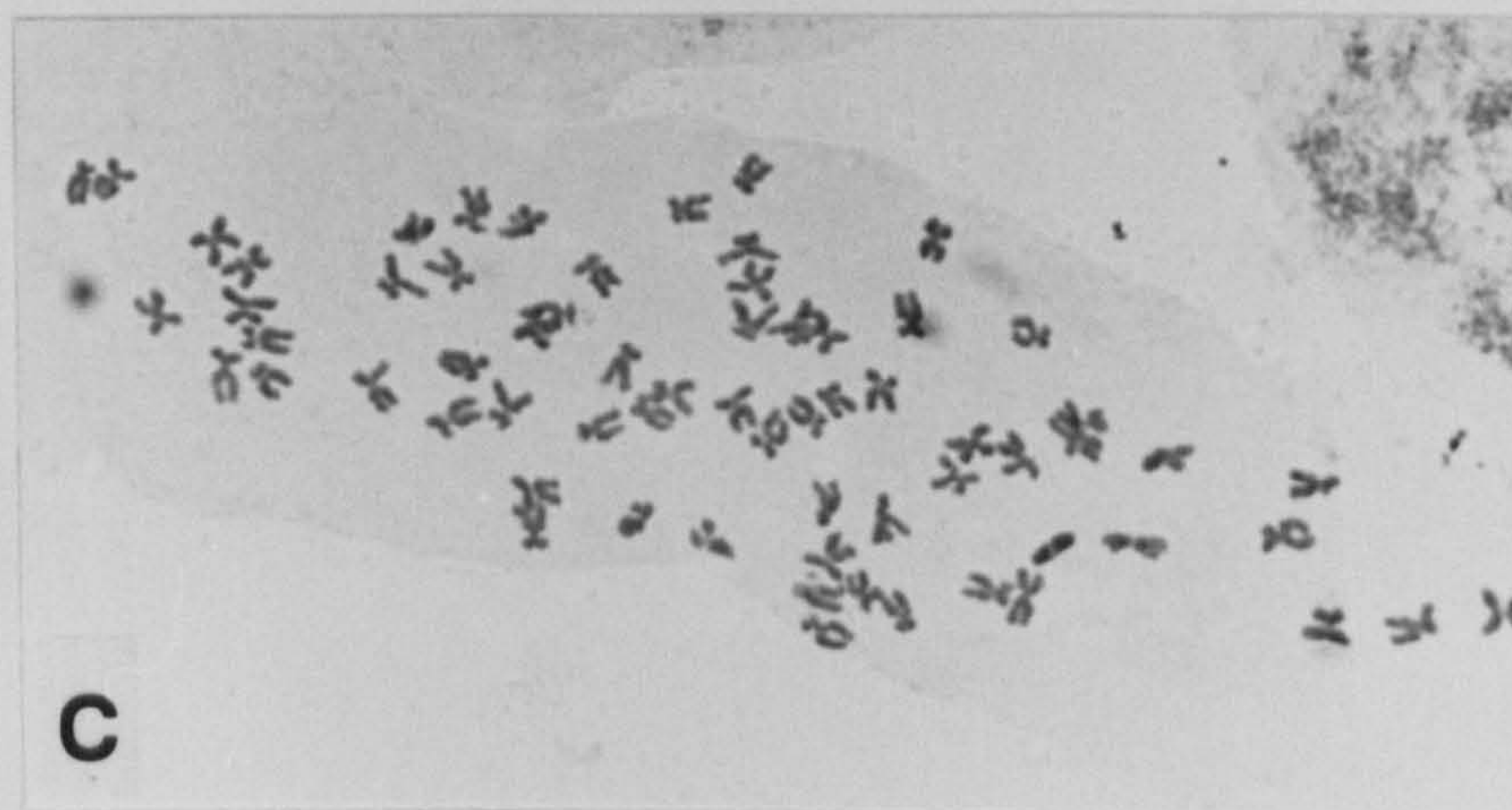
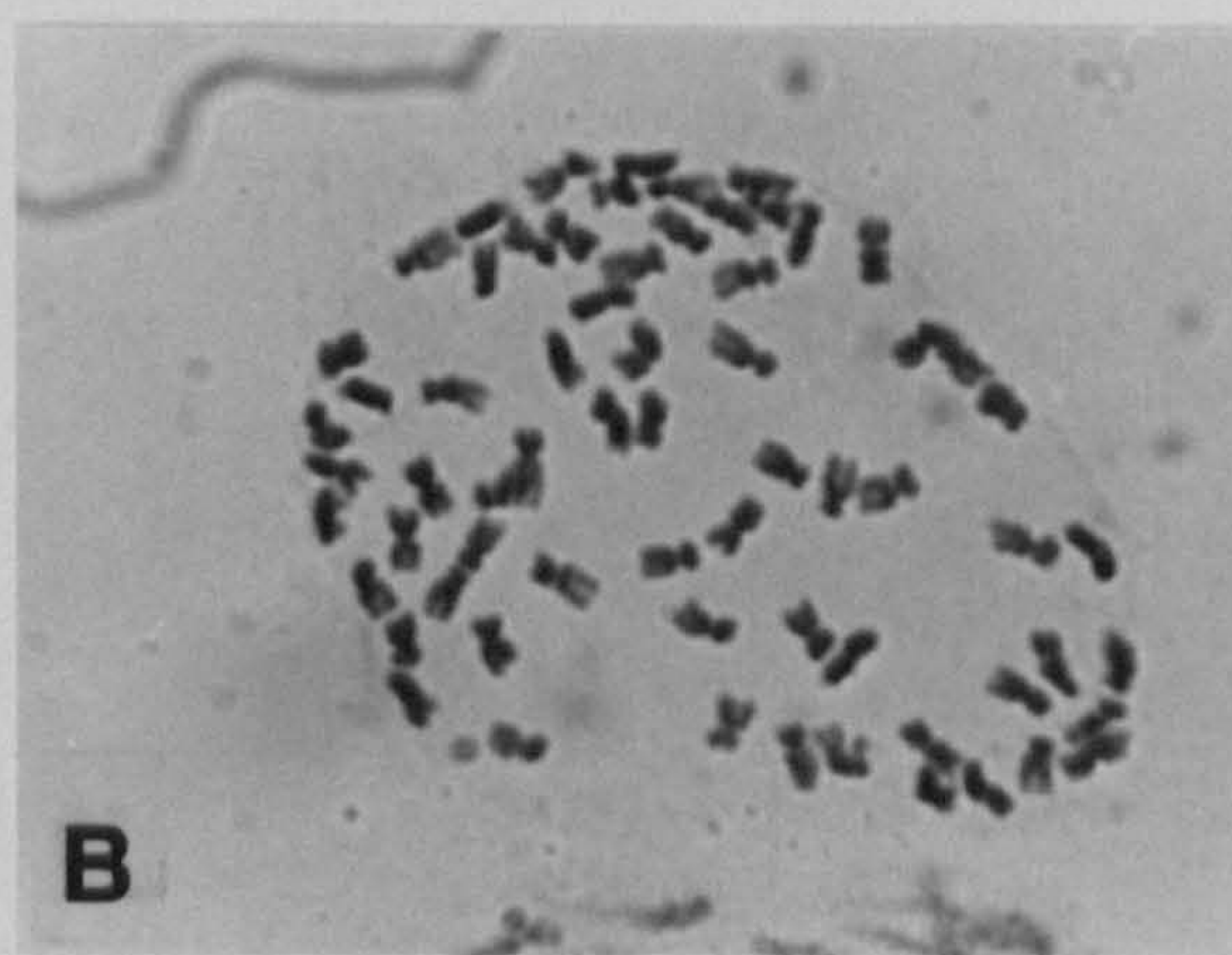
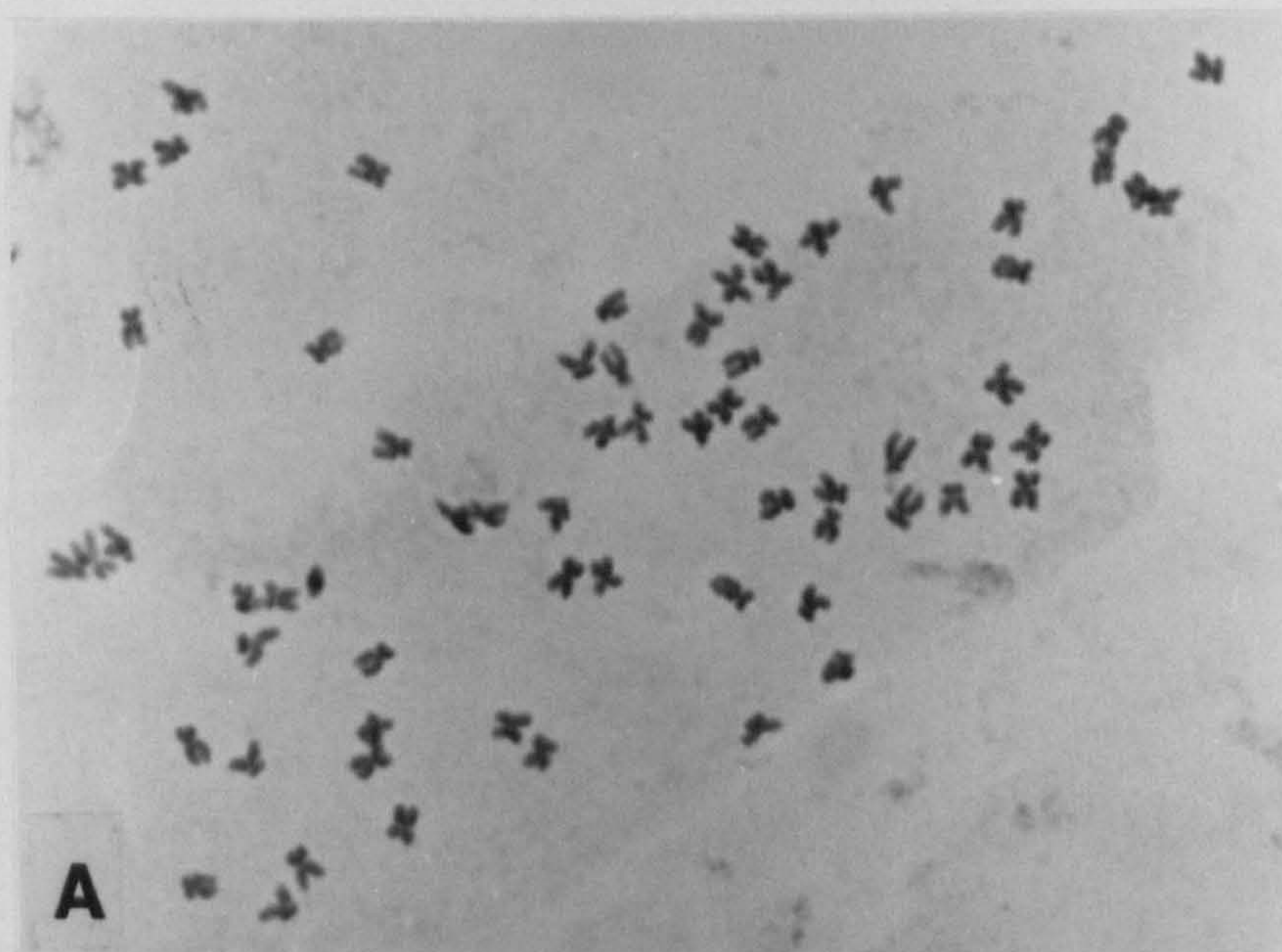


Plate 5.4 Hexaploid mitosis

- A** *R. japonica* x *R. sachalinensis* natural hybrid
P49 $2n = 66$
- B** Artificial *R. japonica* x *R. japonica* var *compacta* hybrid P 76
 $2n = 66$
- C** *R. japonica* x *R. sachalinensis* natural hybrid P40 $2n = 66$

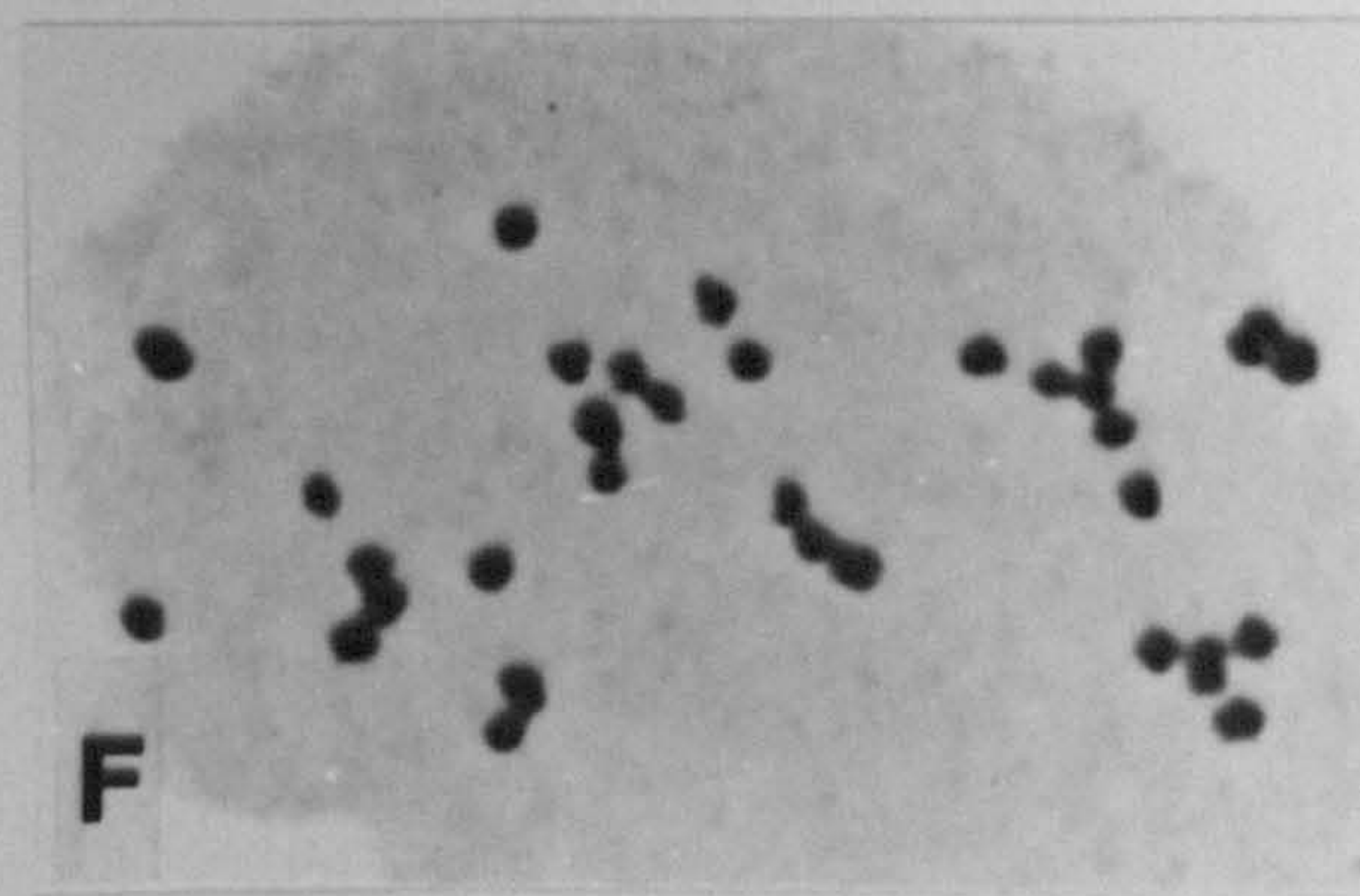
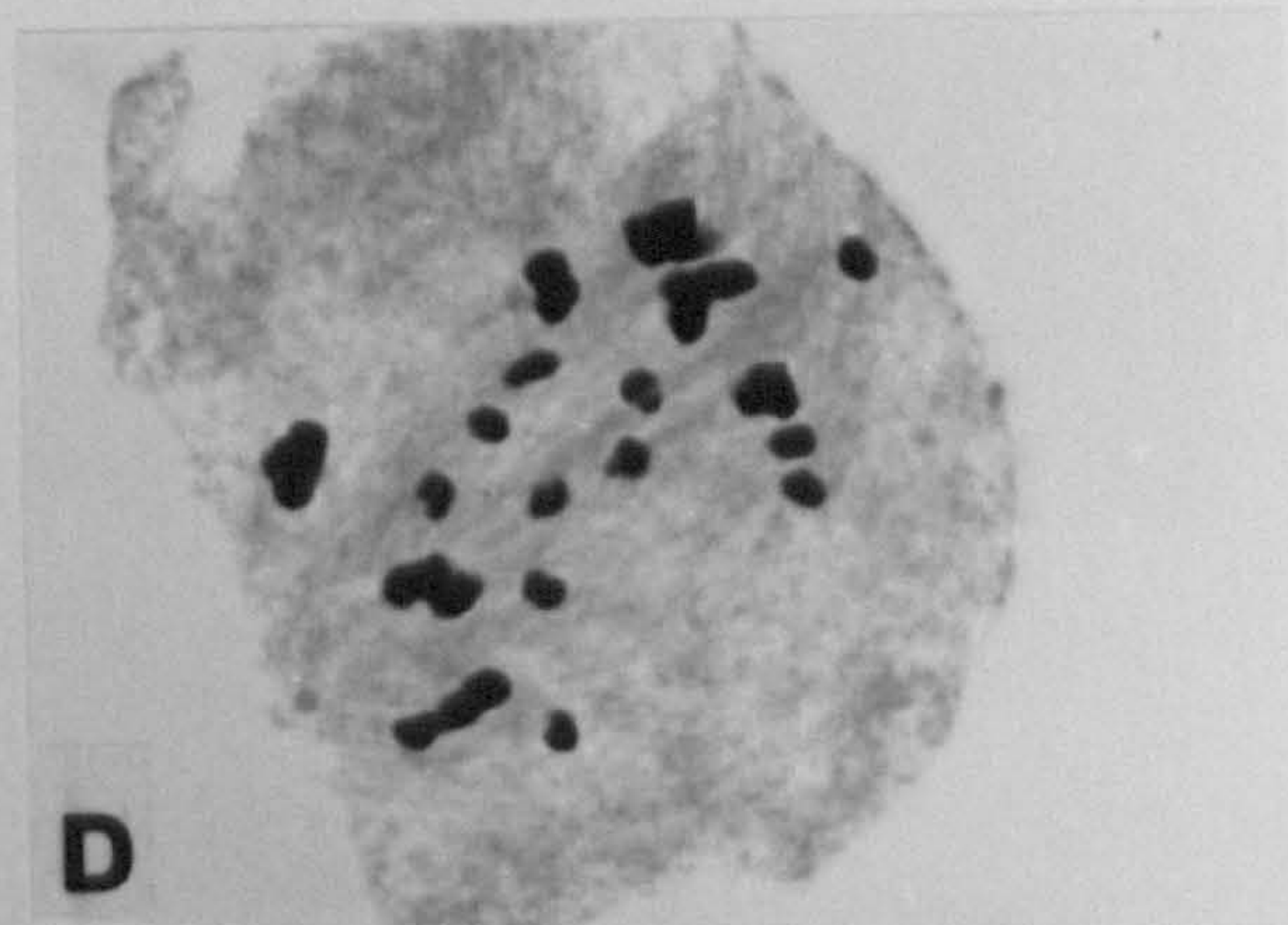
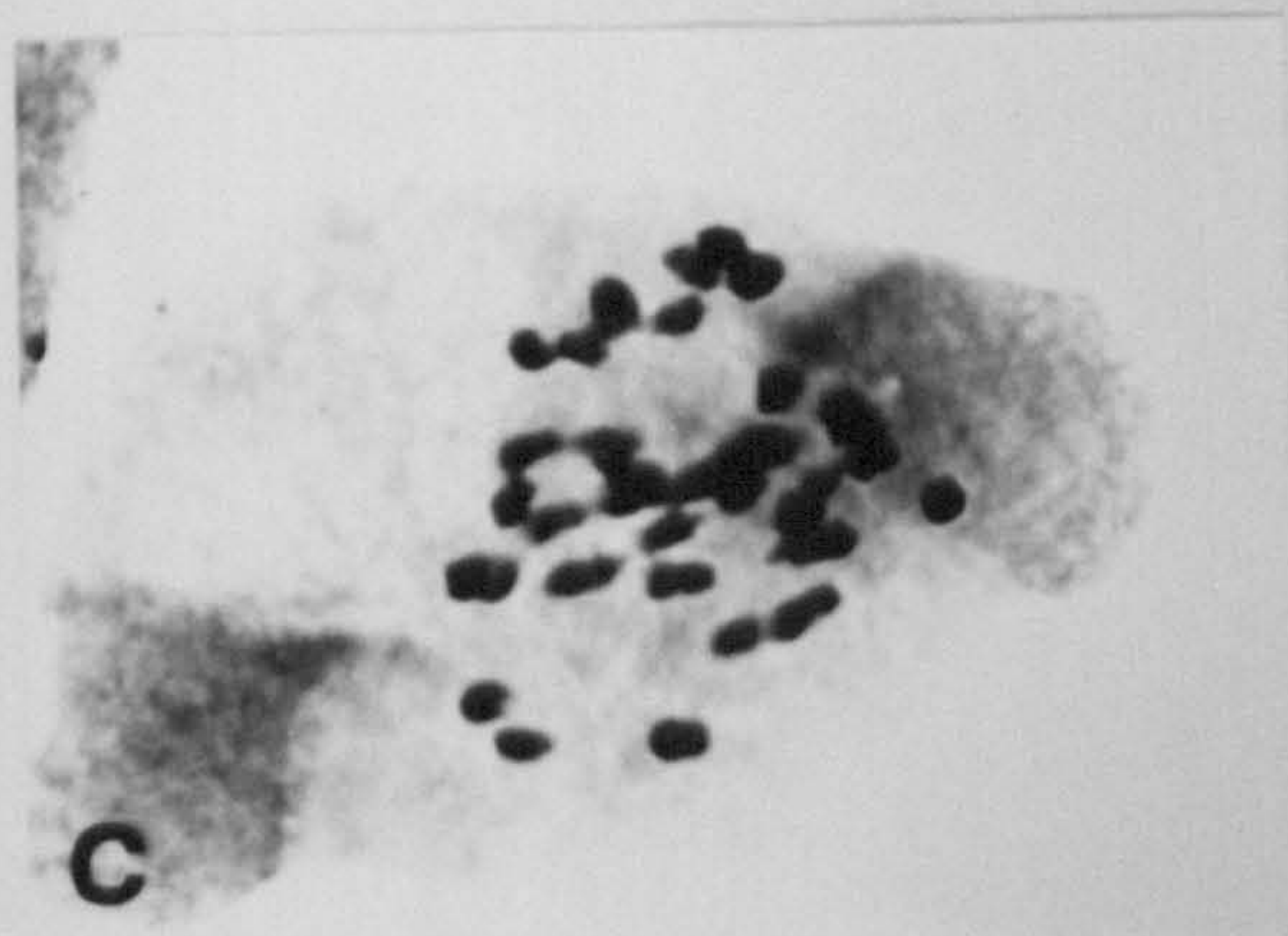
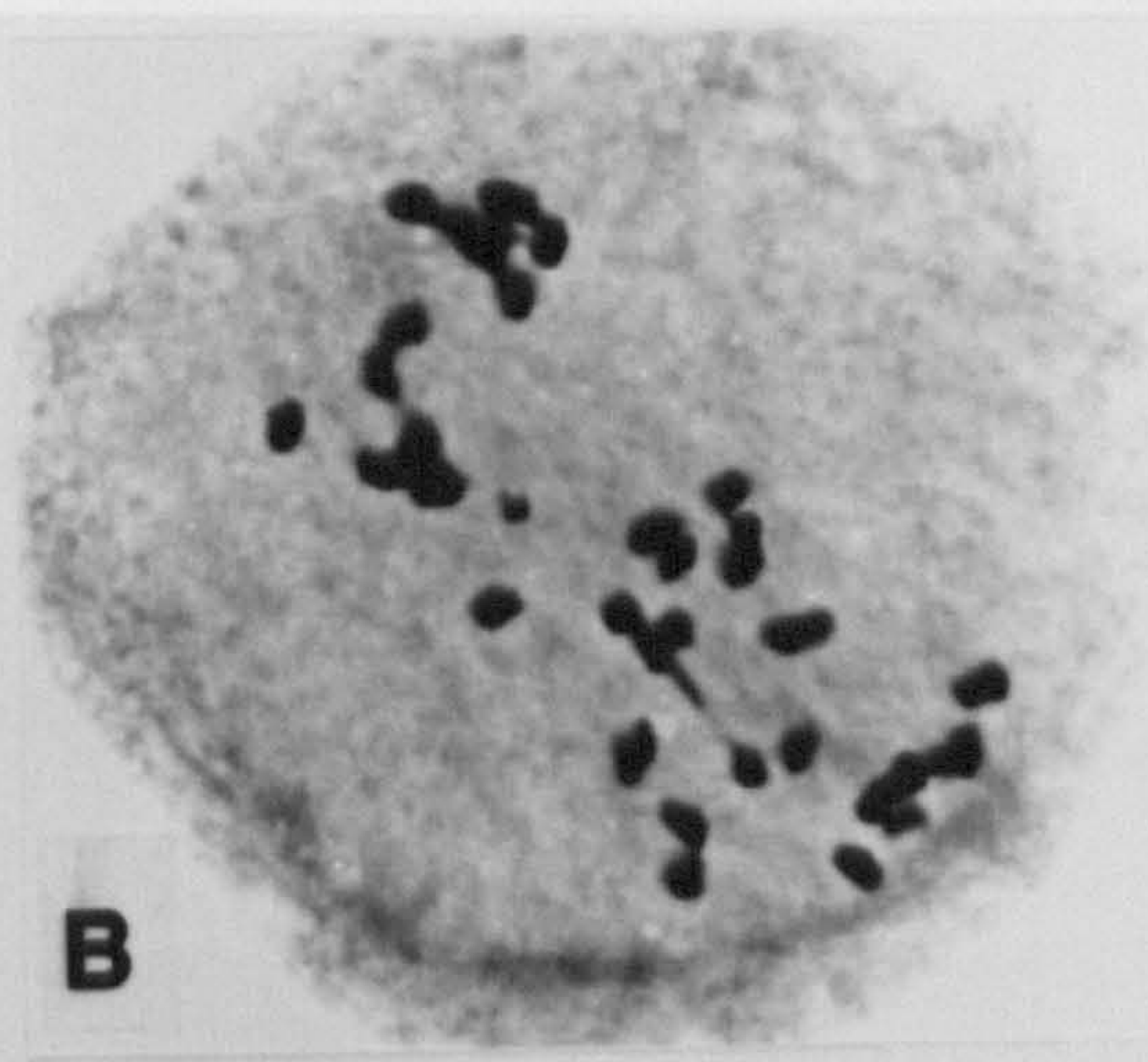
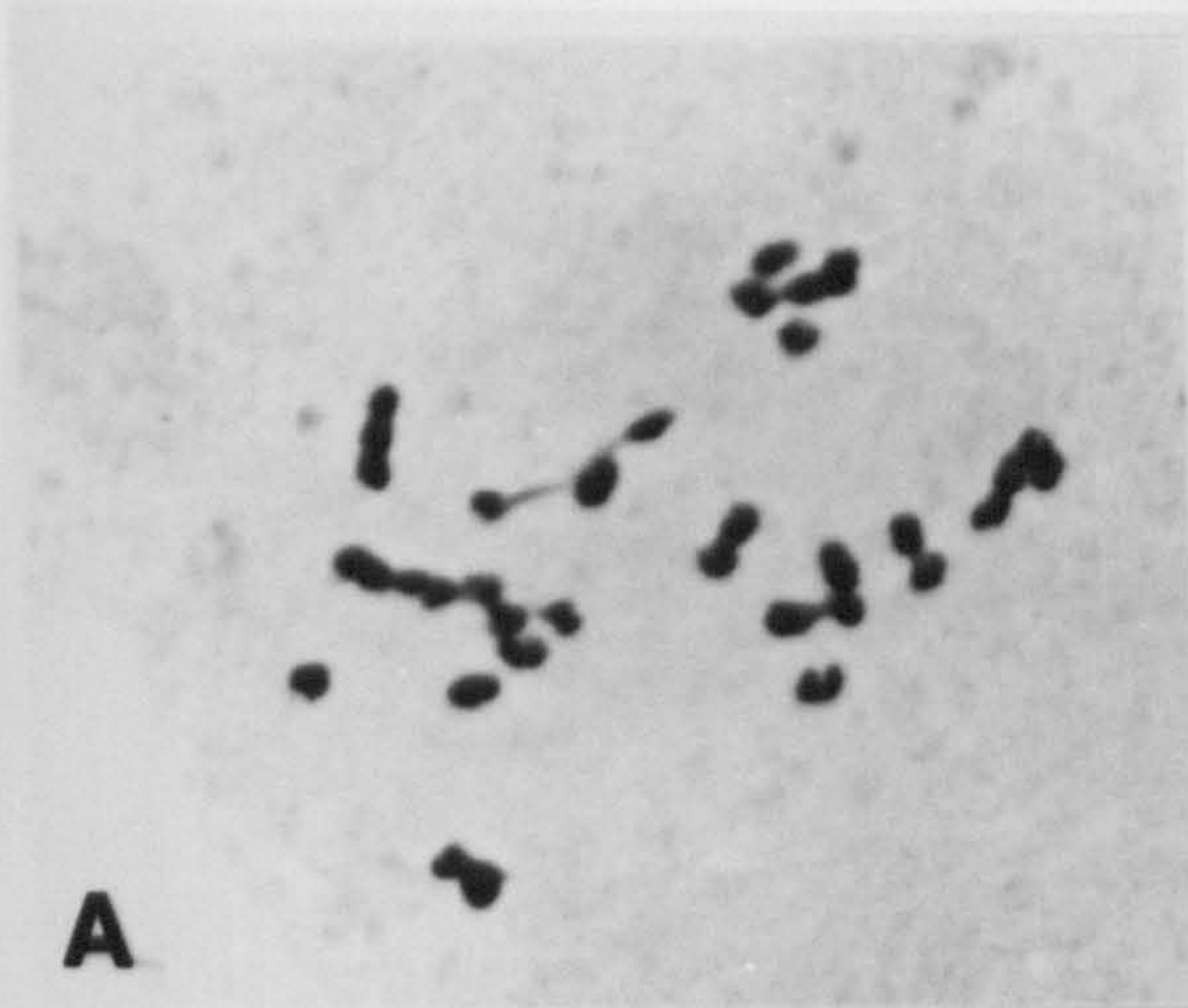


Plate 5.5

Triploid Meiosis

(tetraploid *Reynoutria* x *F. baldschuanica* P102a
 $2n = 32$)

A 18I 7II

B 17I 6II 1III

C 14I 9II

D 13I 5II 3III

E 16I 8II

F 20I 6II

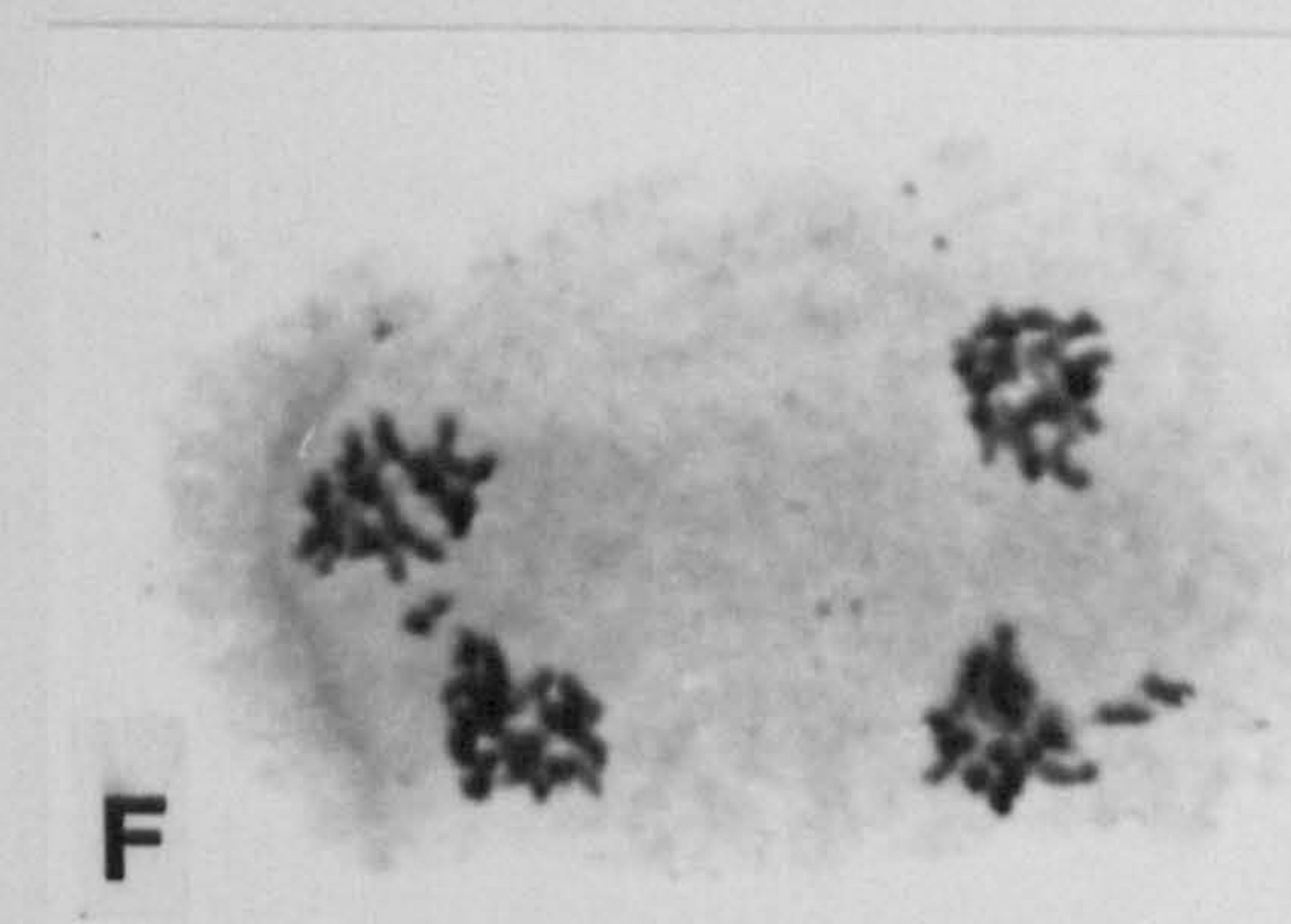
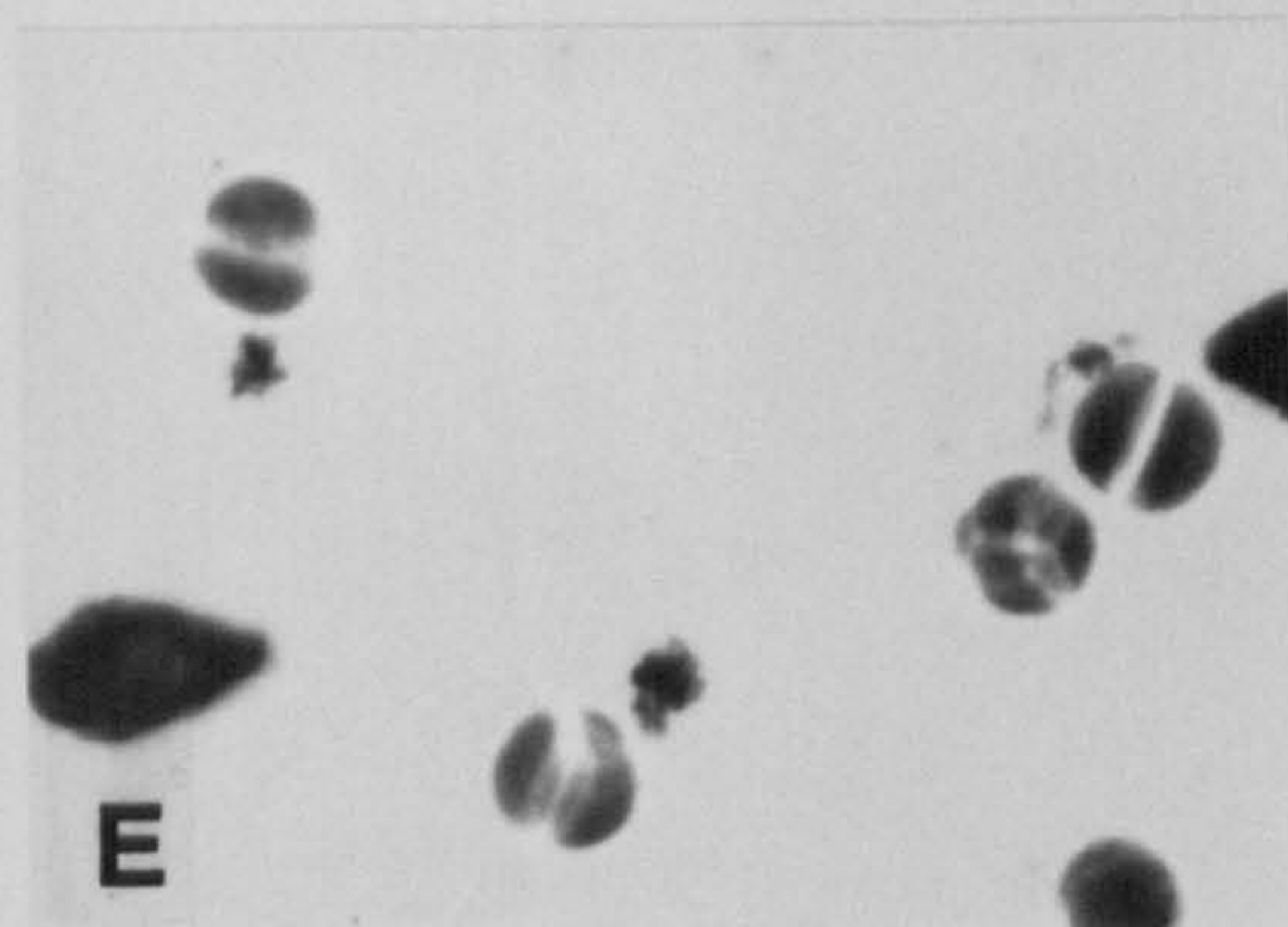
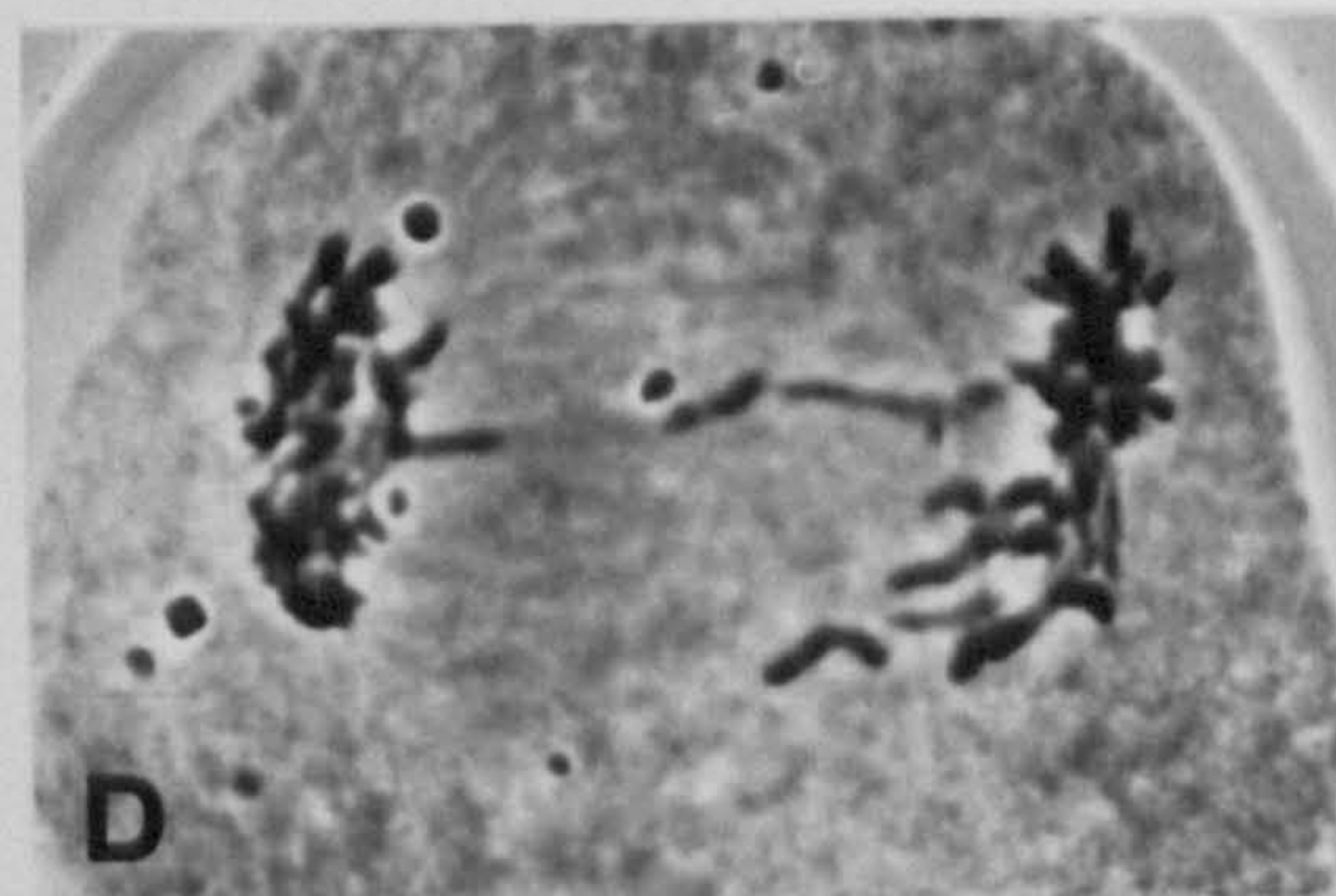
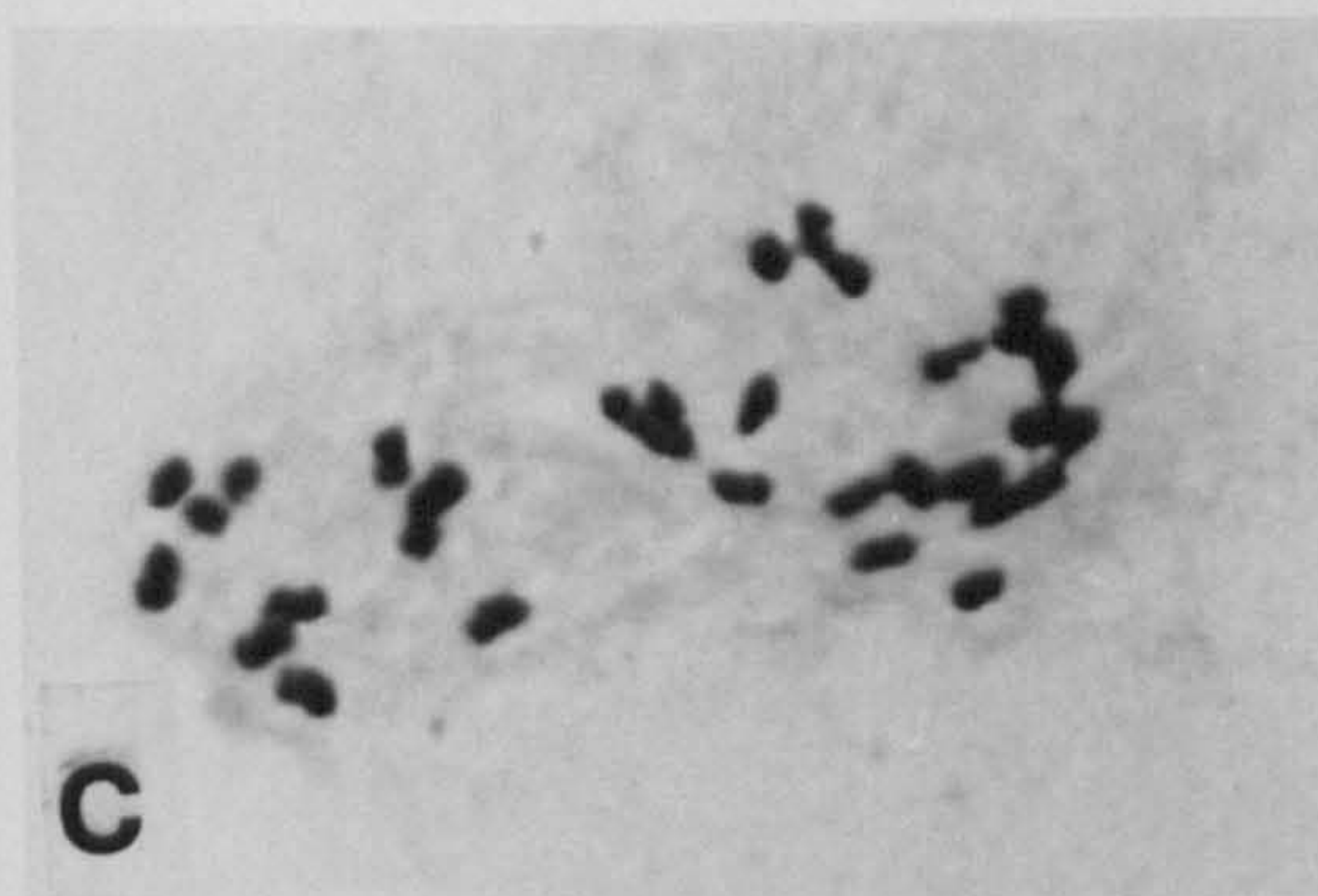
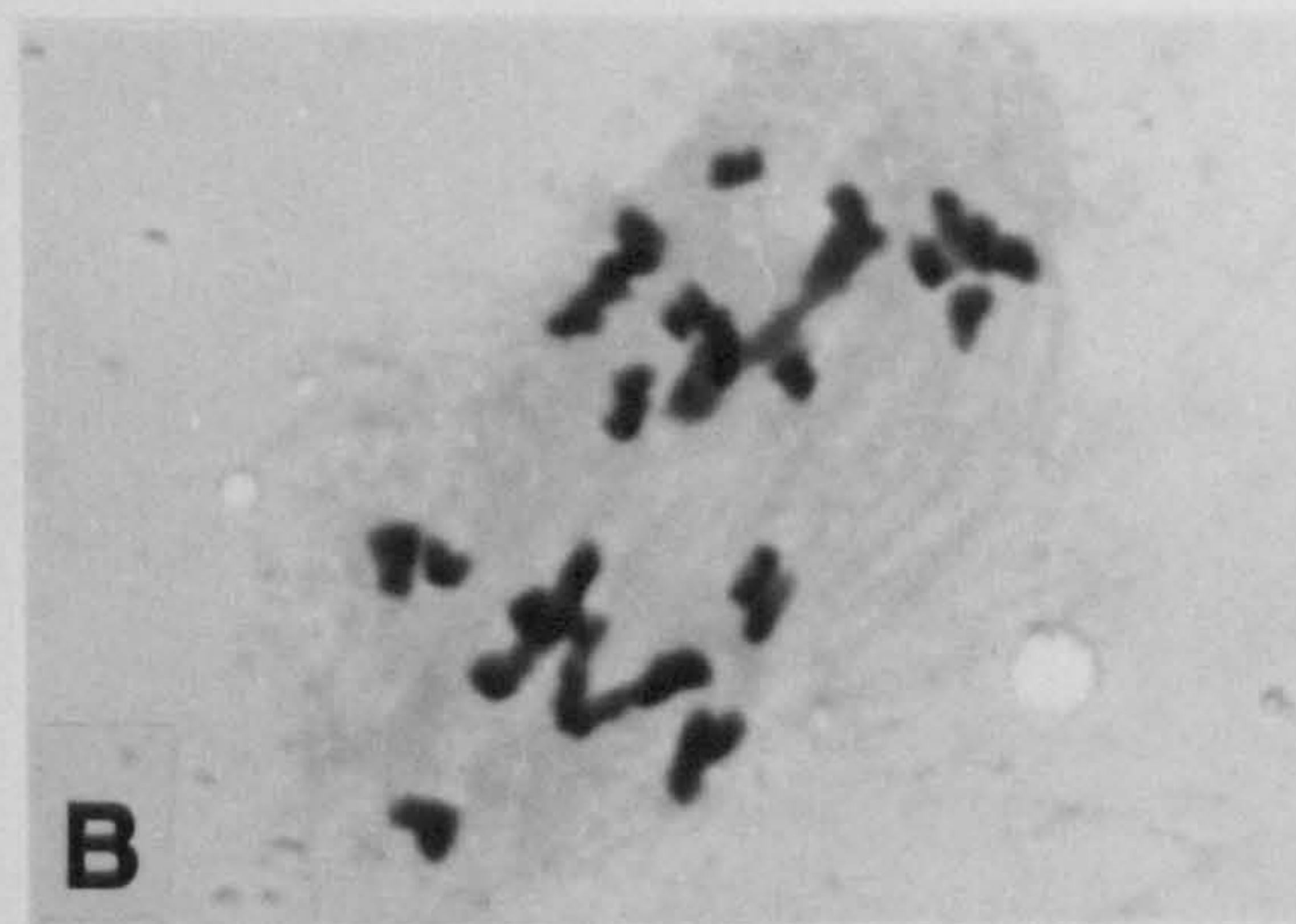
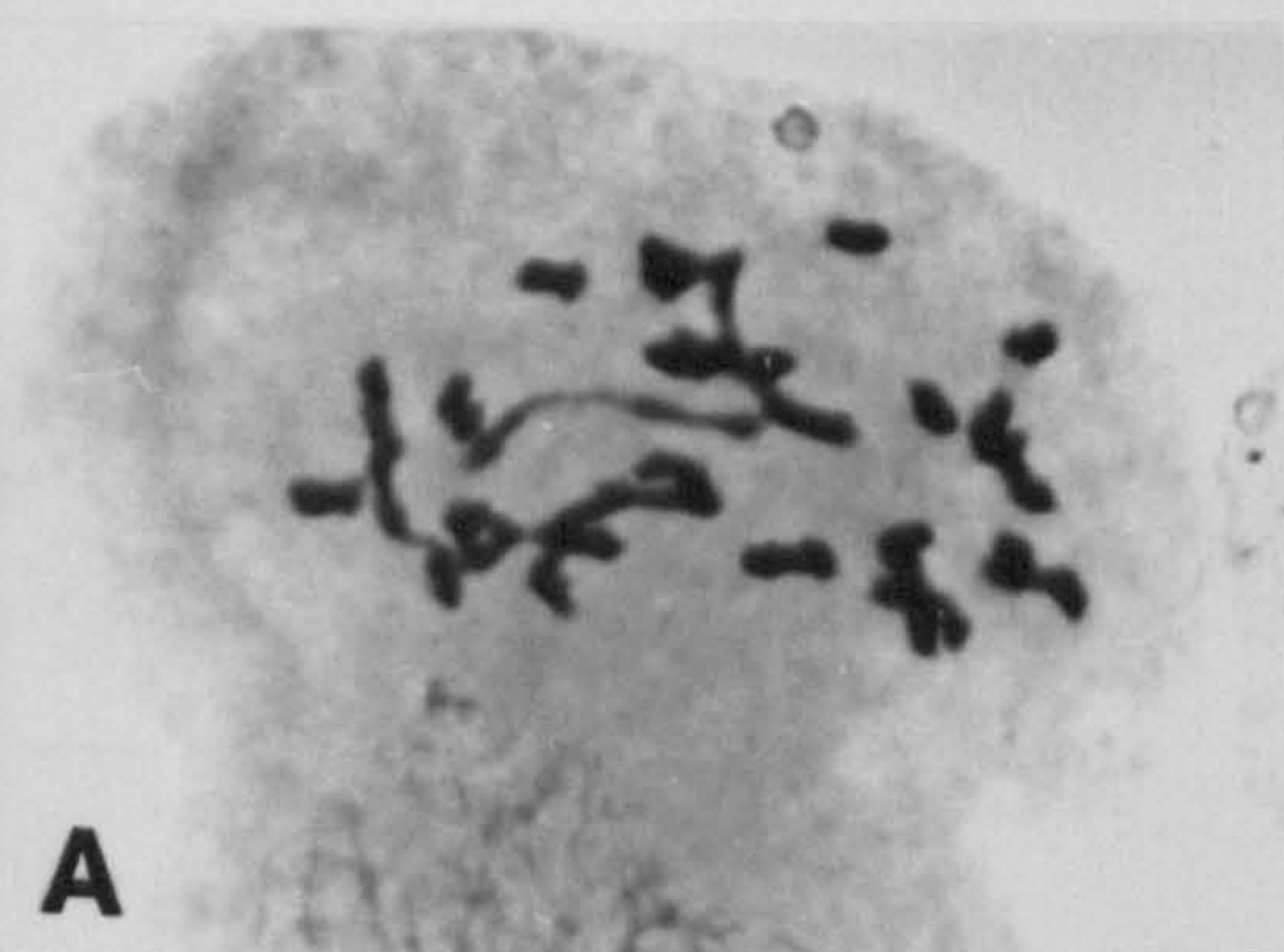


Plate 5.6 **Meiosis in *R. sachalinensis***
x *F. baldschuanica* hybrids $2n = 32$

- | | | | |
|----------|---|----------|------------------------------------|
| A | P.101c 20I 3II 2III | B | P101c 21I 4II 1III |
| C | P101 22I 5II | D | P101 telophase I with bridge |
| E | P101c post telophase II
showing restitution diads
and micronuclei | F | P101c telophase 2 with
laggards |

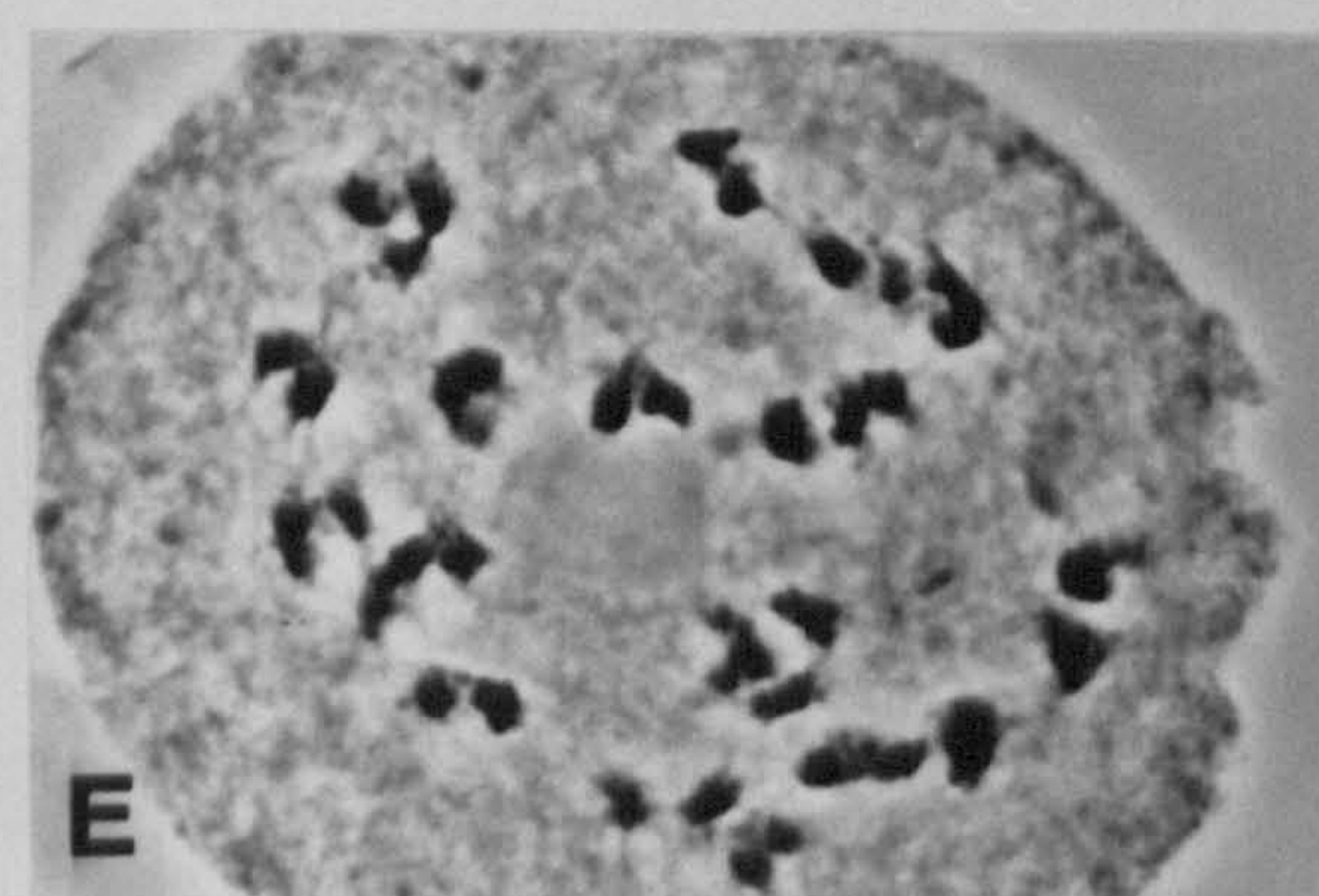
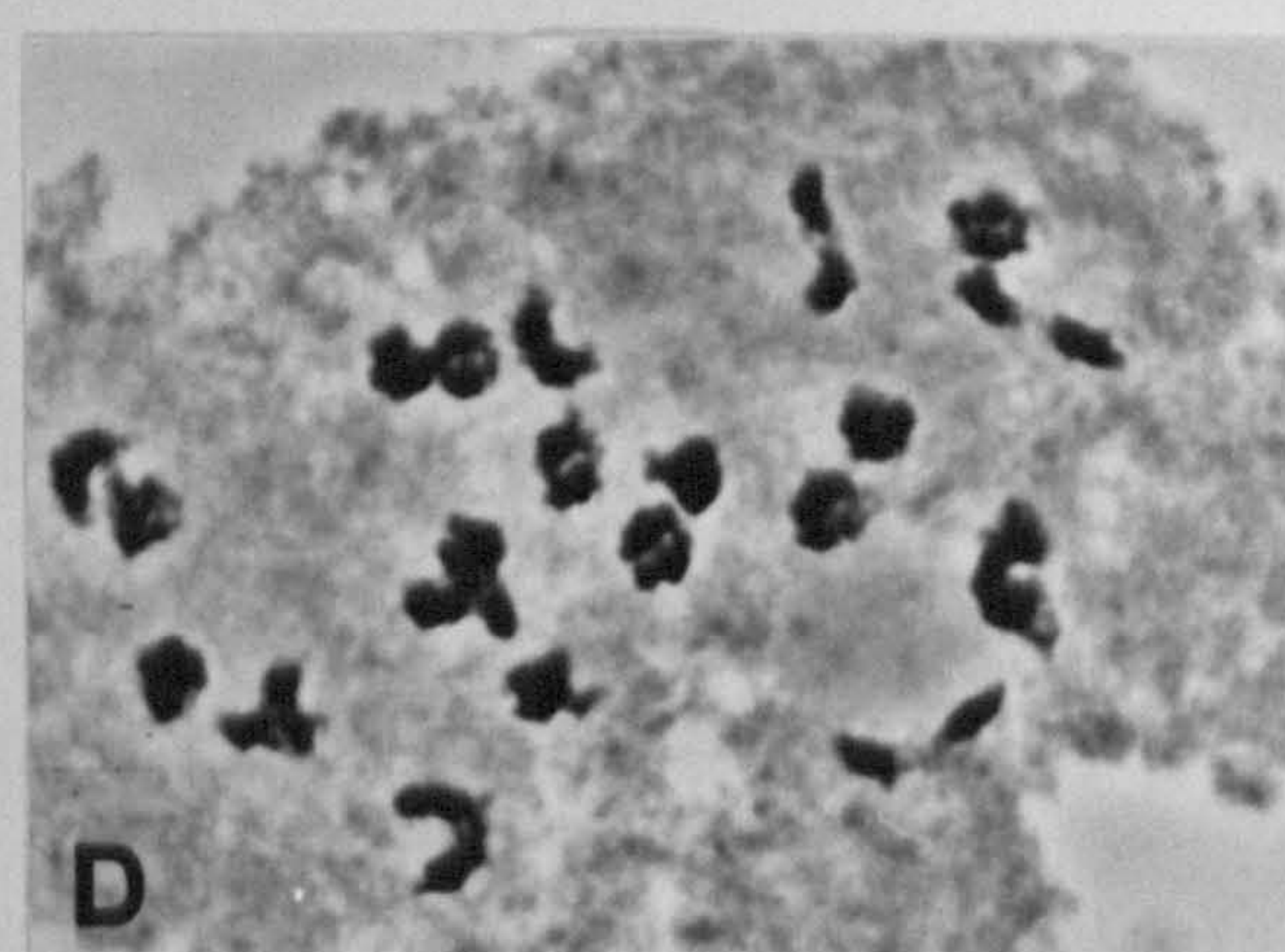
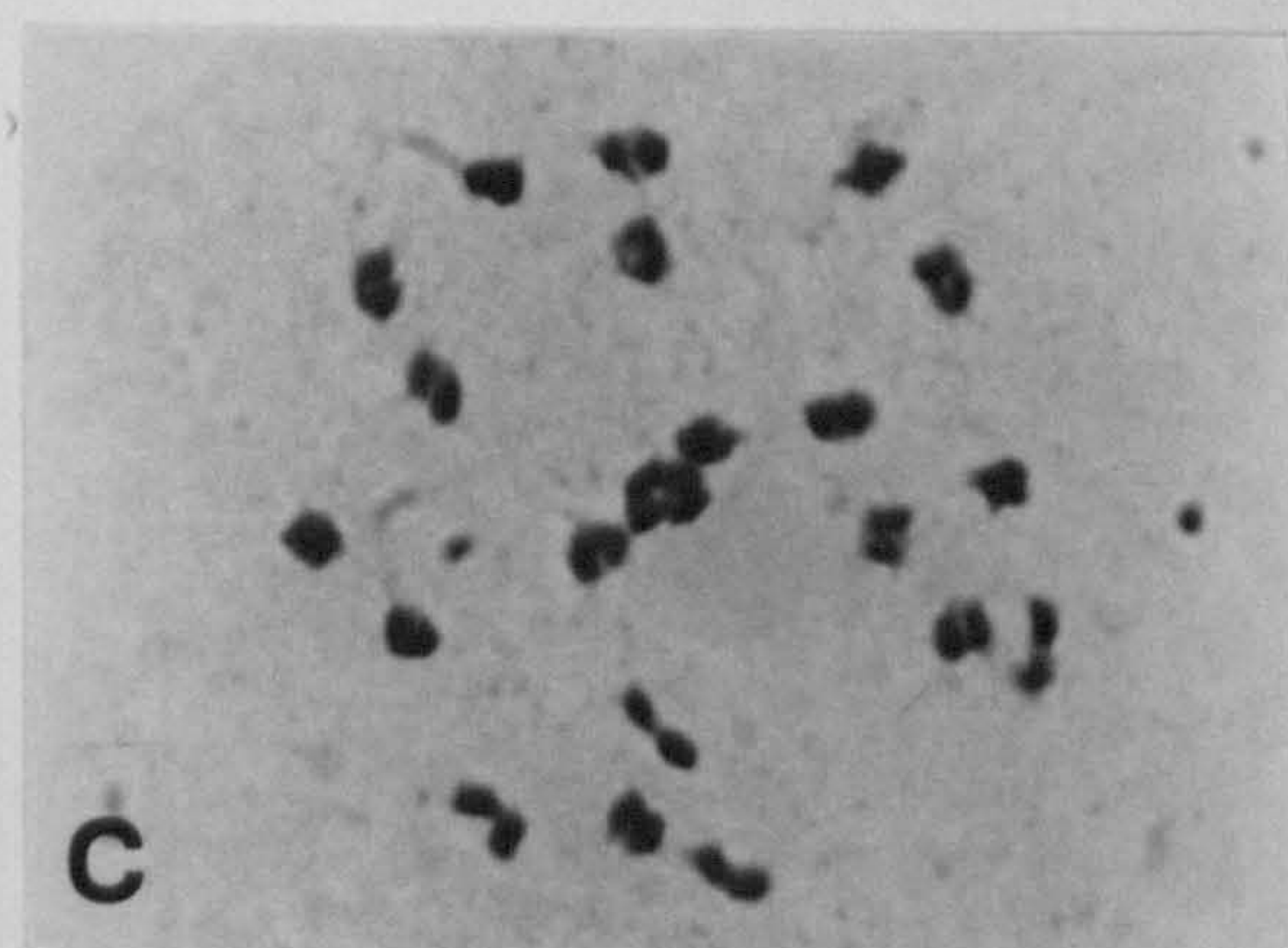
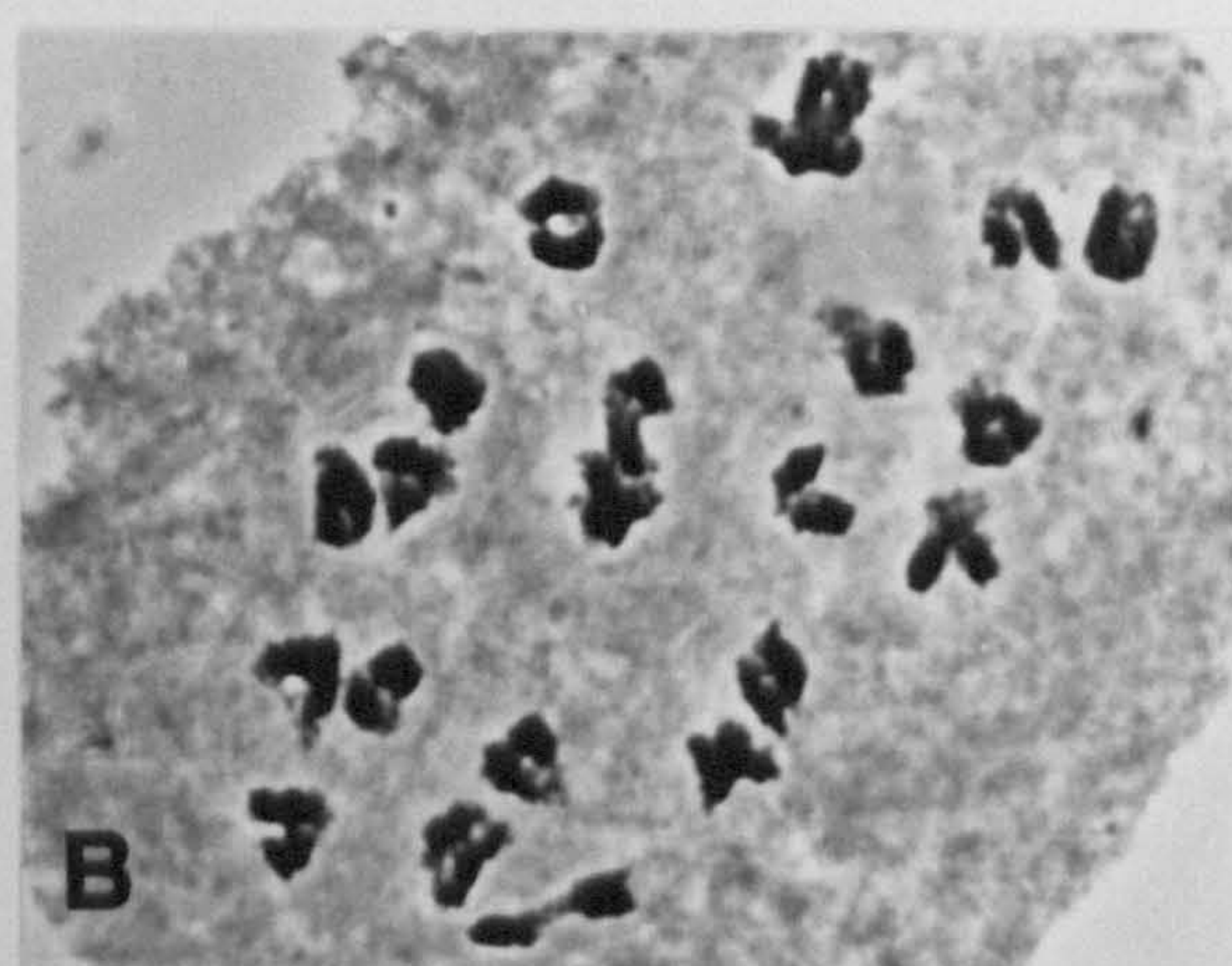
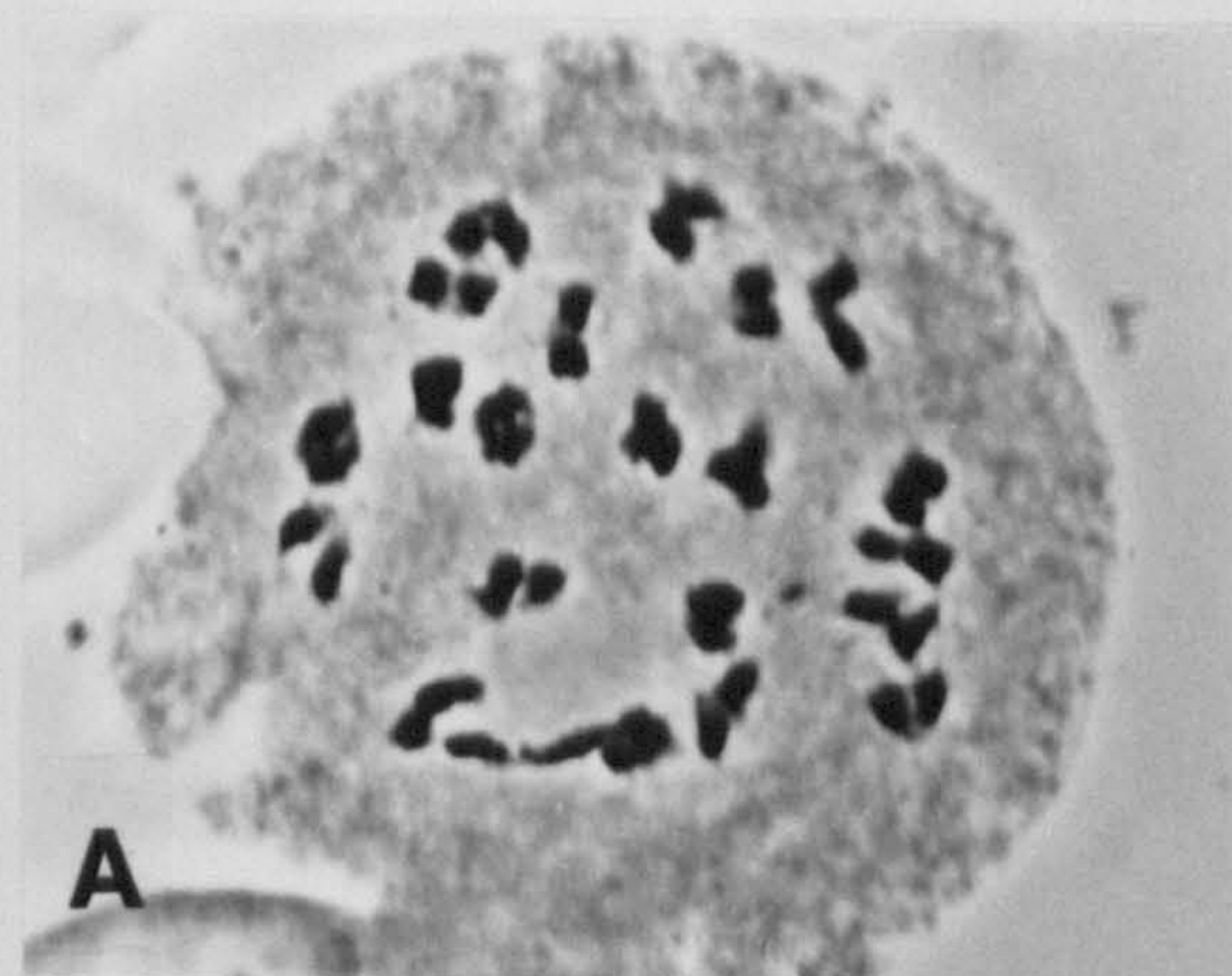


Plate 5.7 Tetraploid *Reynoutria* hybrid meiosis

A,B P13 22II

C P13 20II 1IV

D P13 18I 2IV

E P78a

F P78a 22II

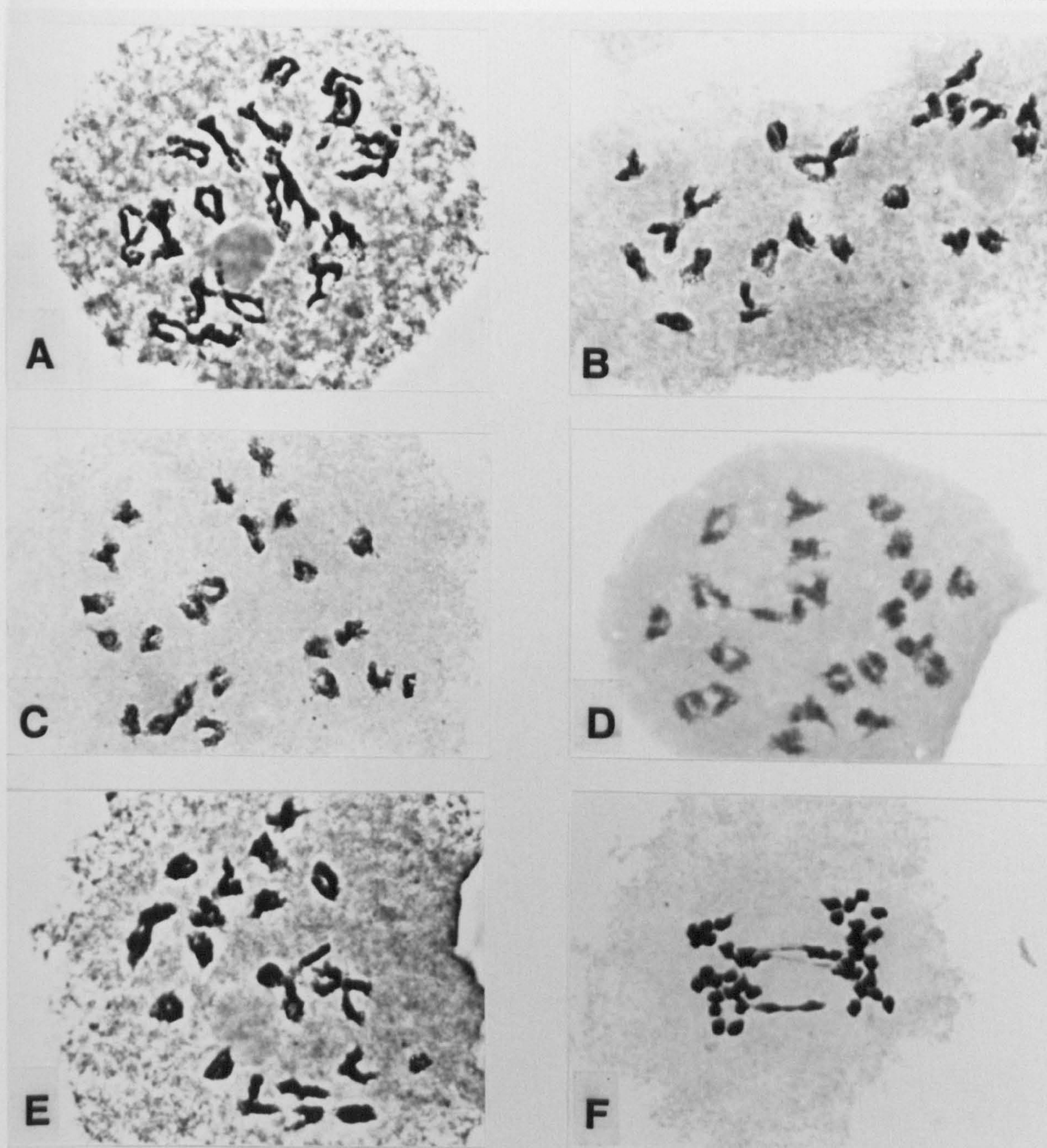


Plate 5.8 Meiosis in an artificial *R.sachalinensis* x *R.japonica* var .*compacta* hybrid P79c

A 18II 2IV

B 2I 19II 2IV

C 20II 1IV

D 22II

E 18II 2IV

F late anaphase I with laggards and bridges

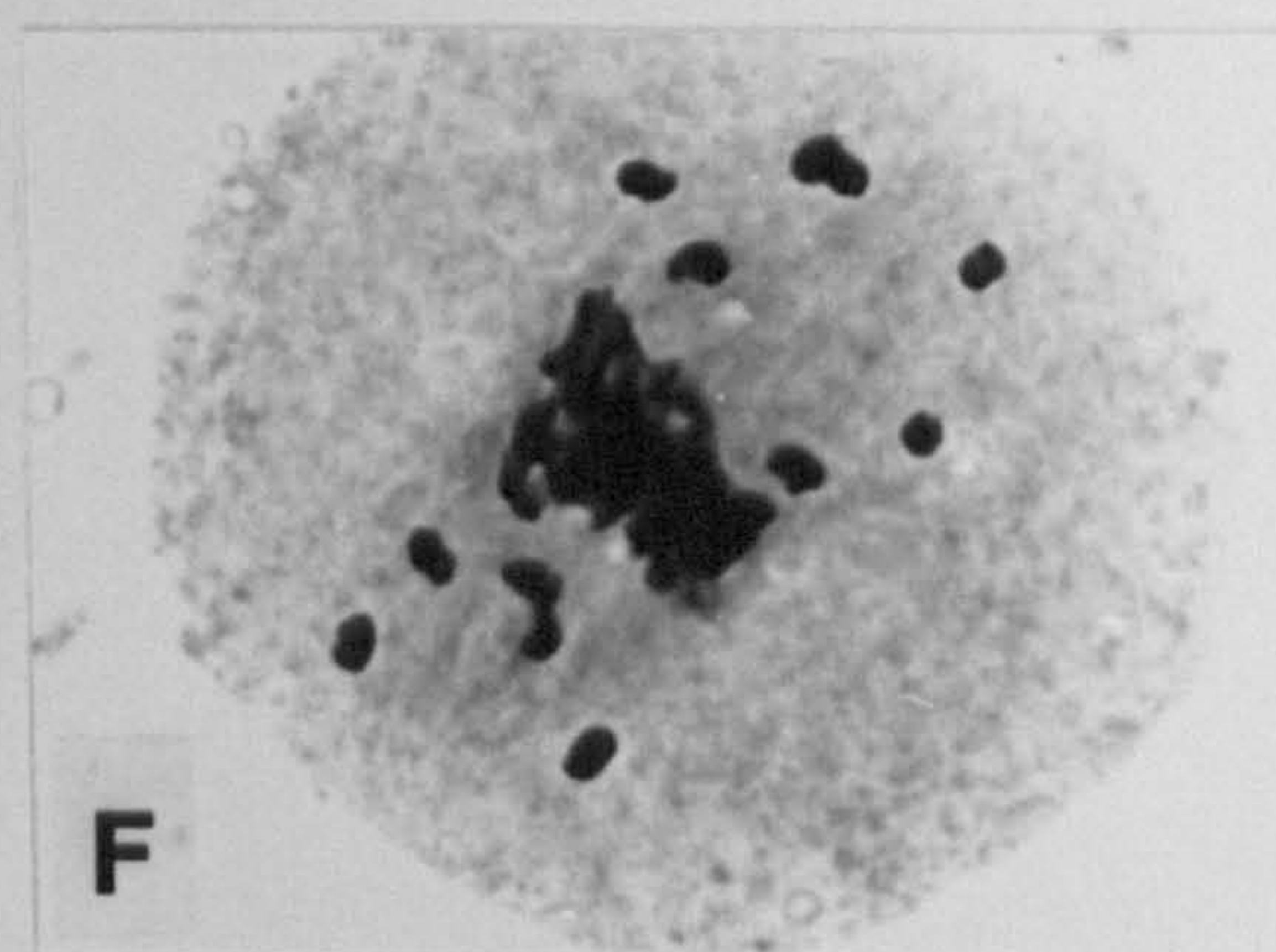
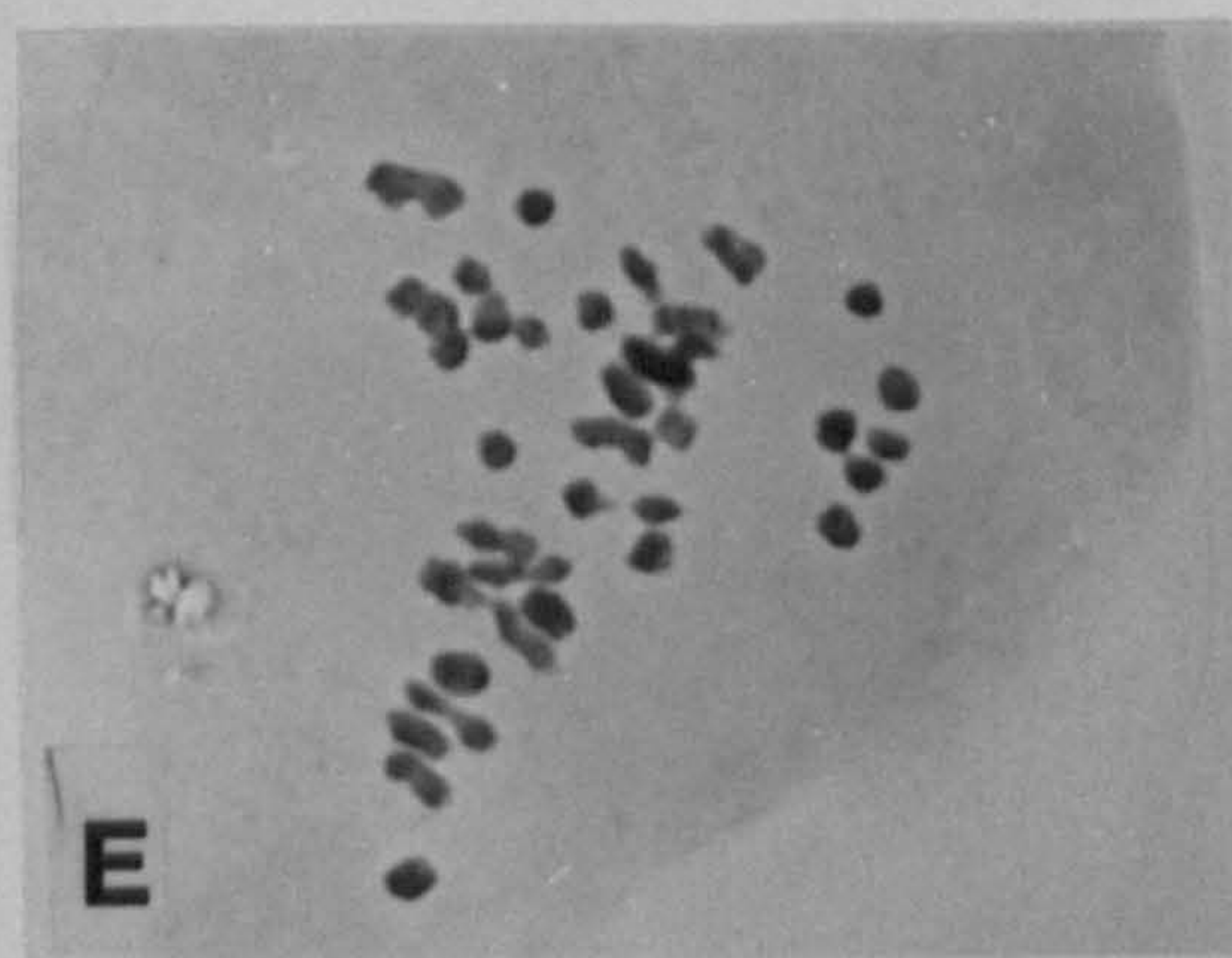
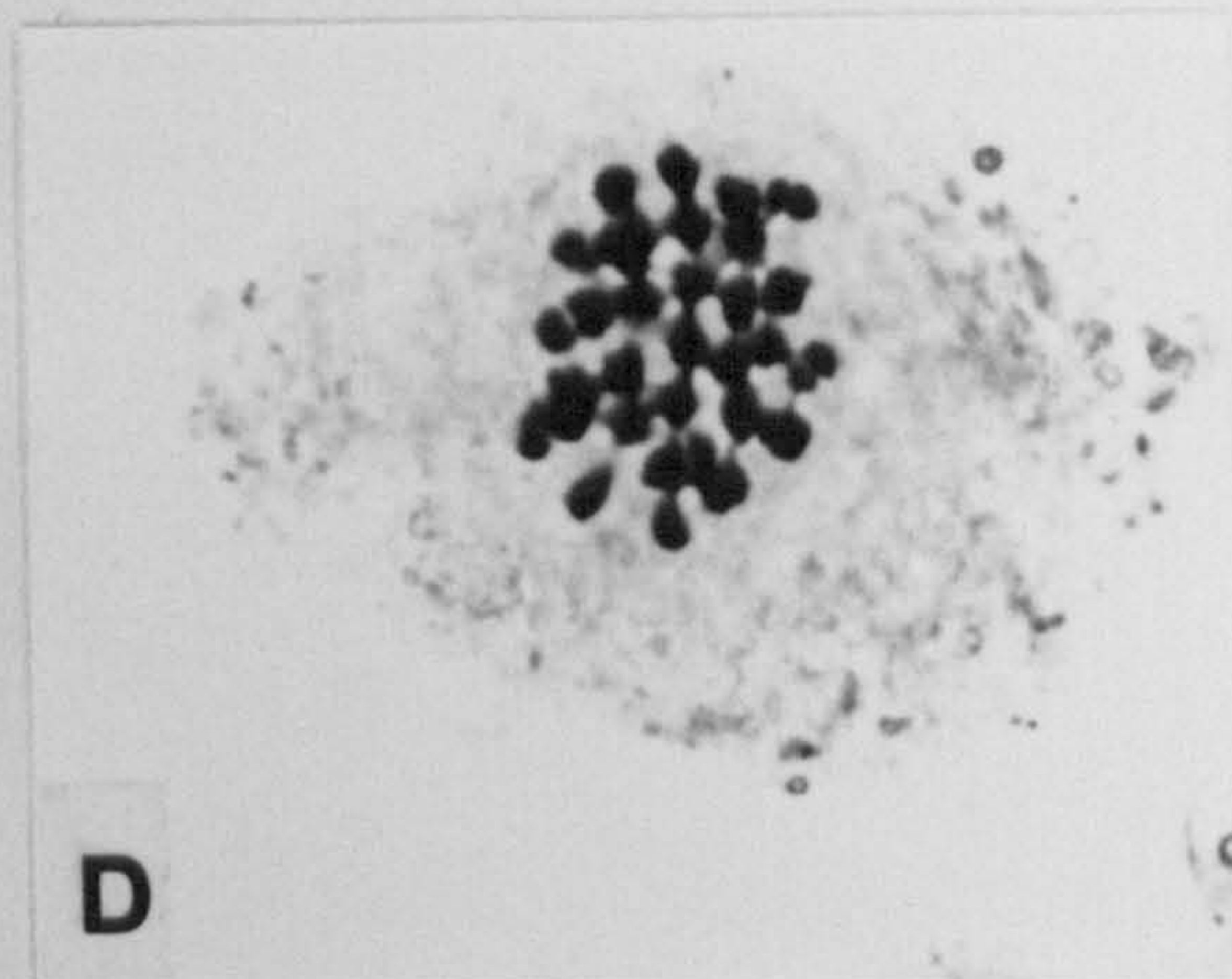
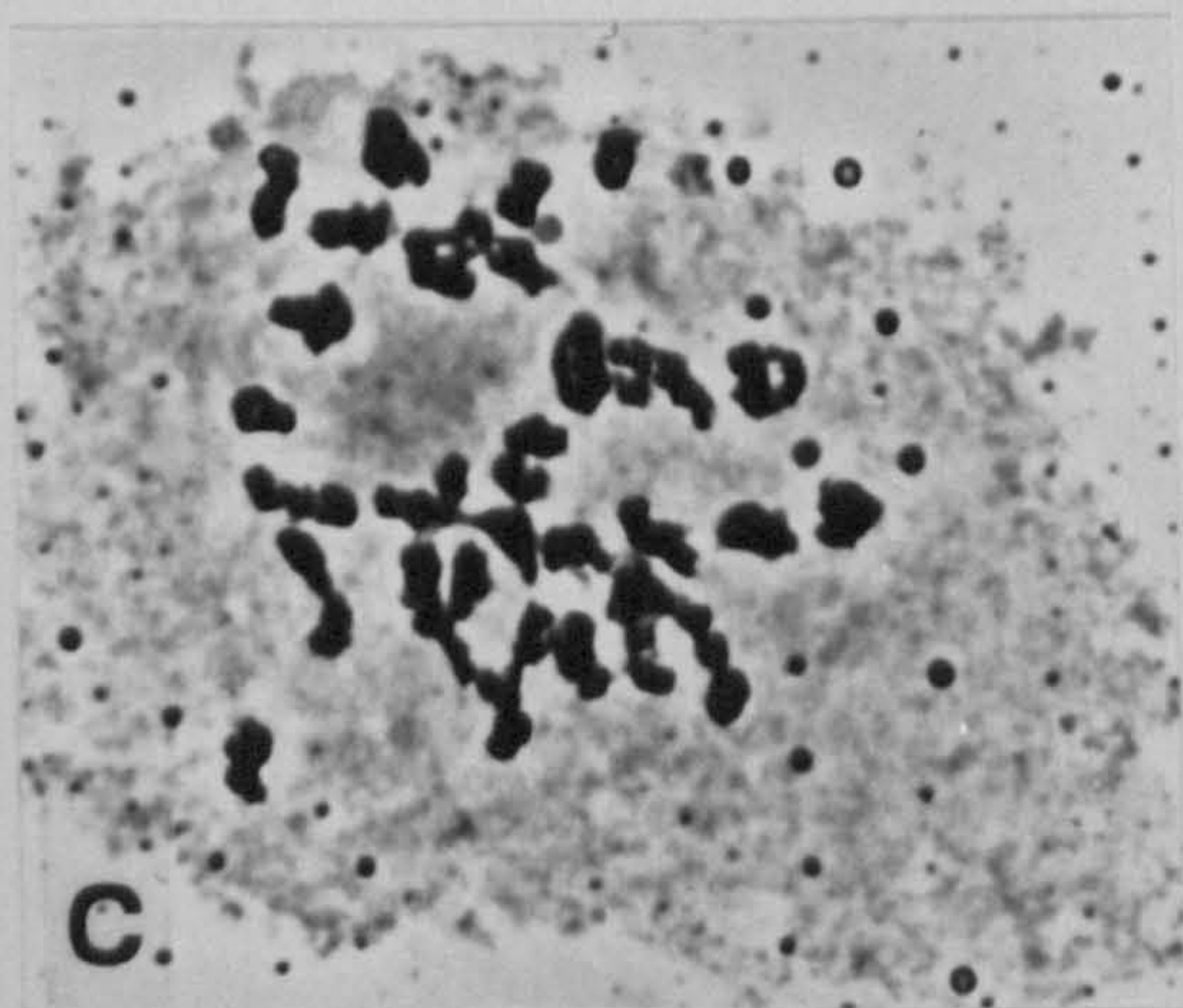
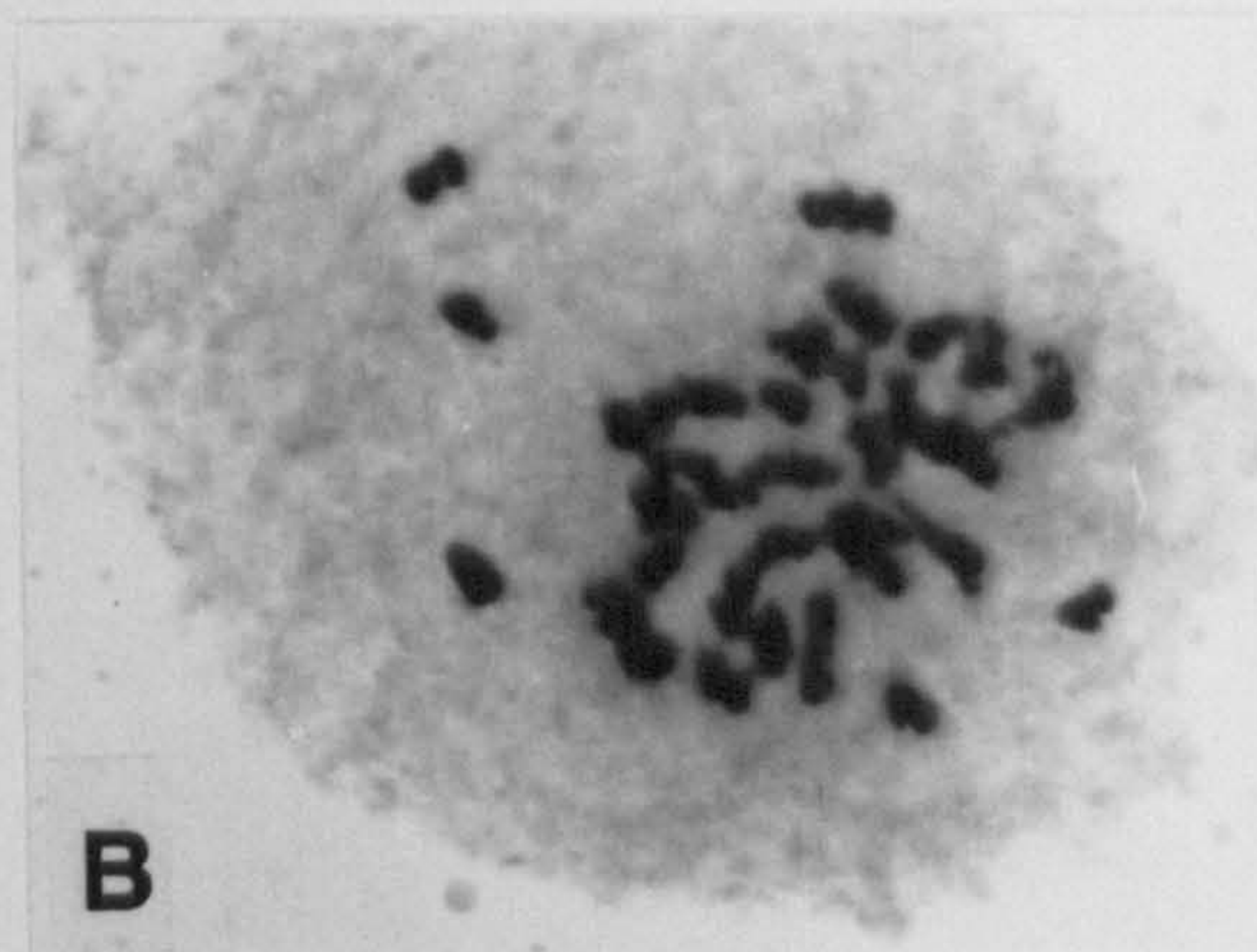
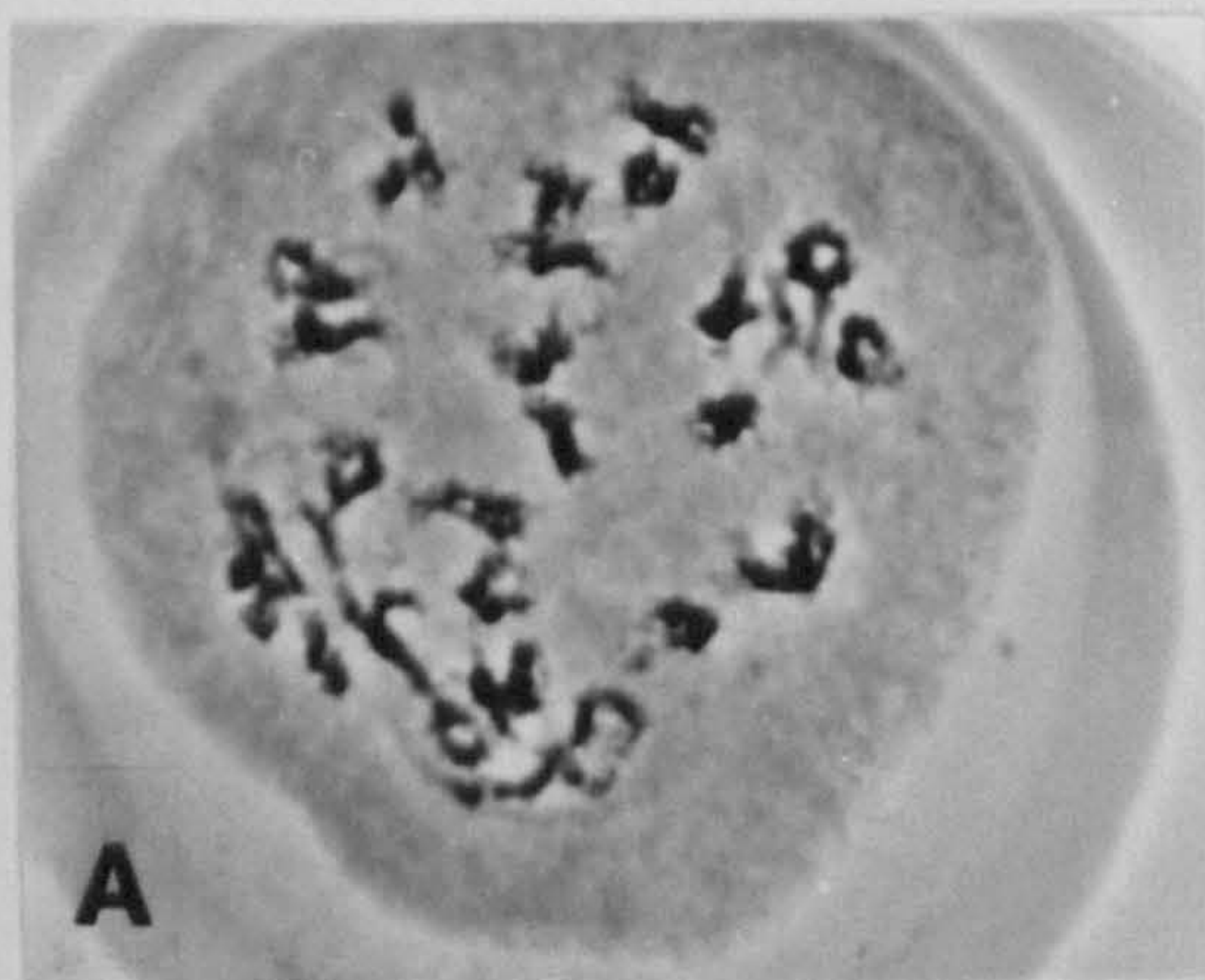


Plate 5.9 Meiosis in *R. japonica* x *F. baldschuanica* hybrids $2n = 54$

A P83b diplotene

B P80d 10I 22II

C P80d 10I 22II

D P156 32 chromosome bodies

E P83b 10I 22II

F P80d with 10I off the spindle

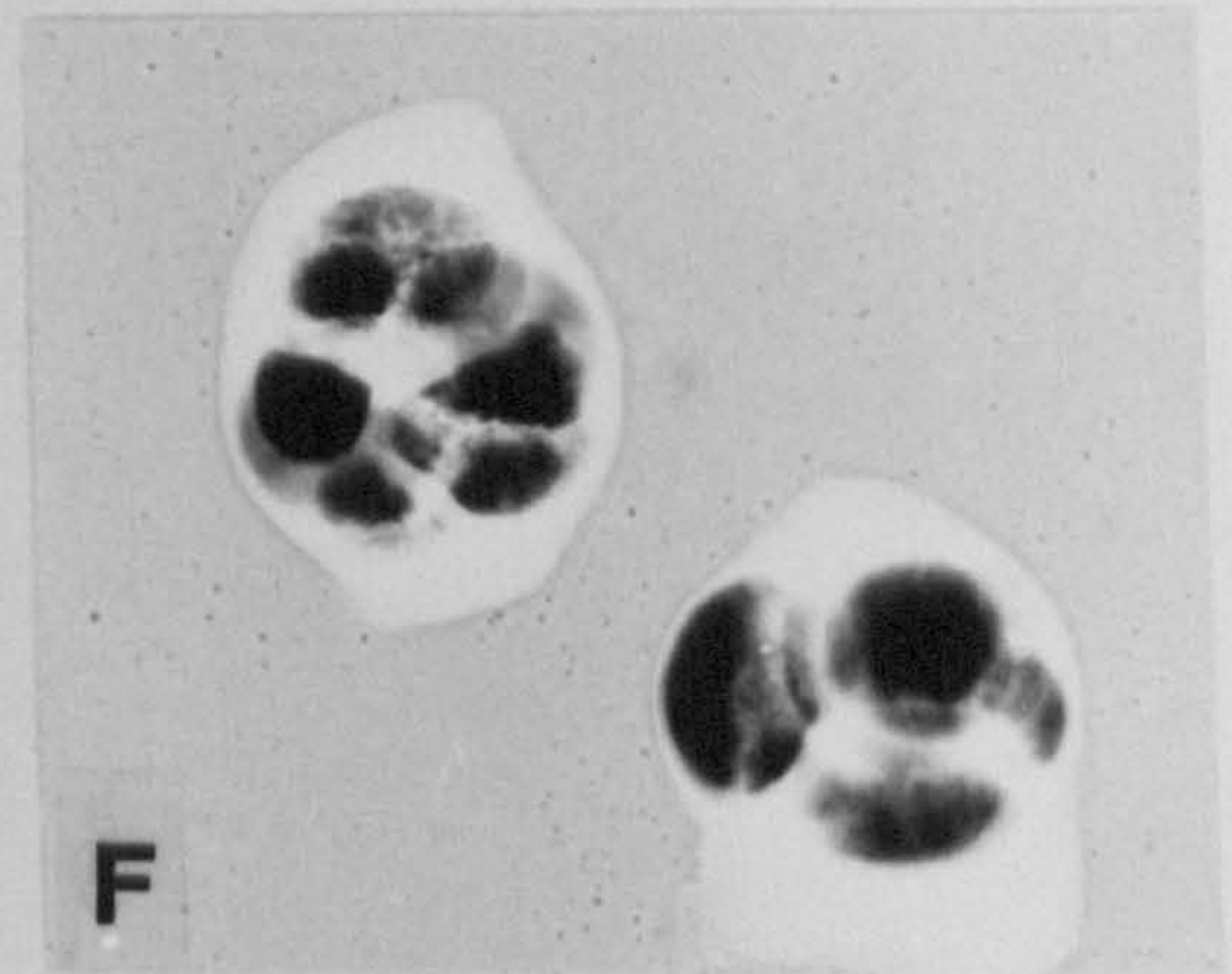
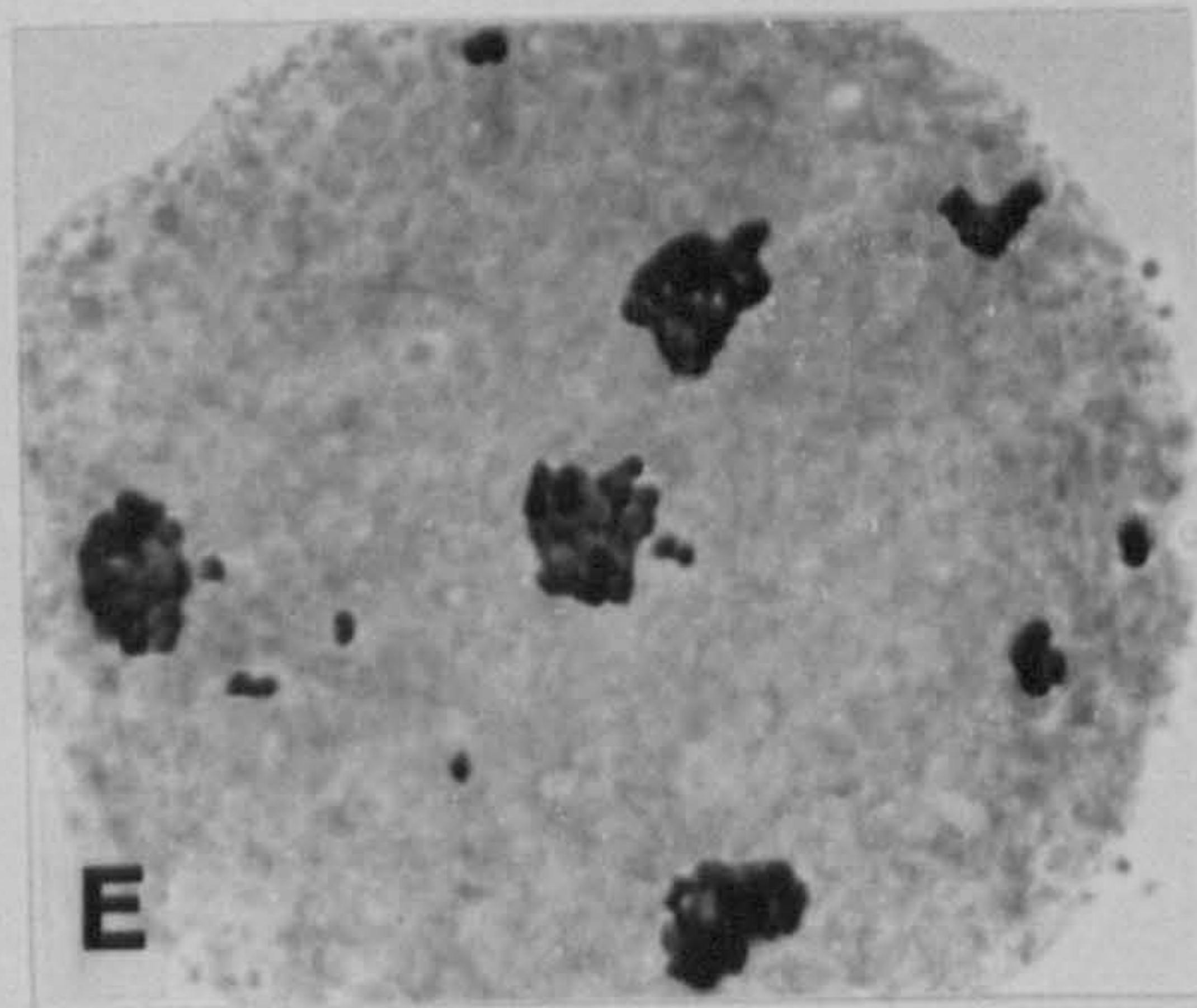
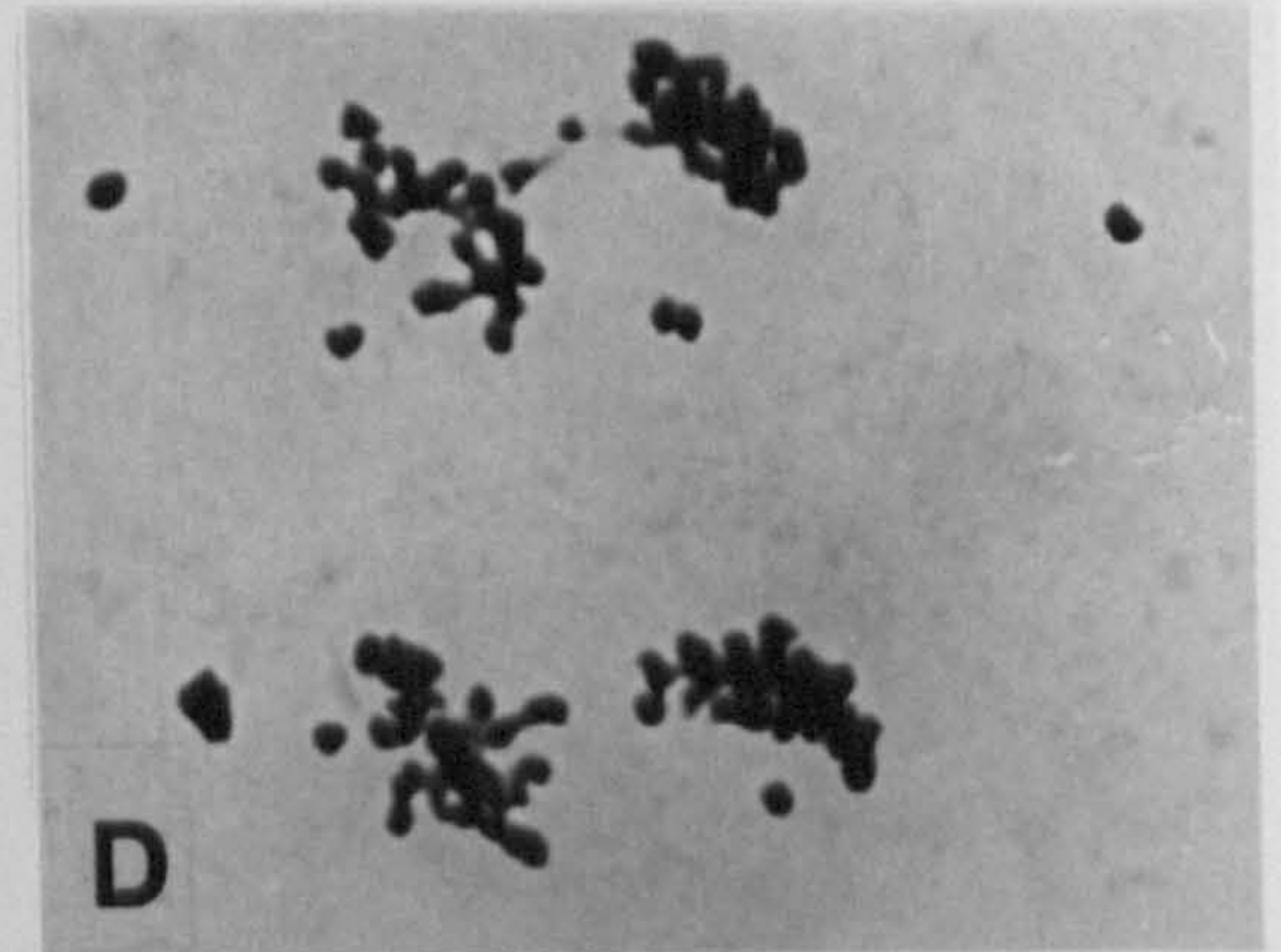
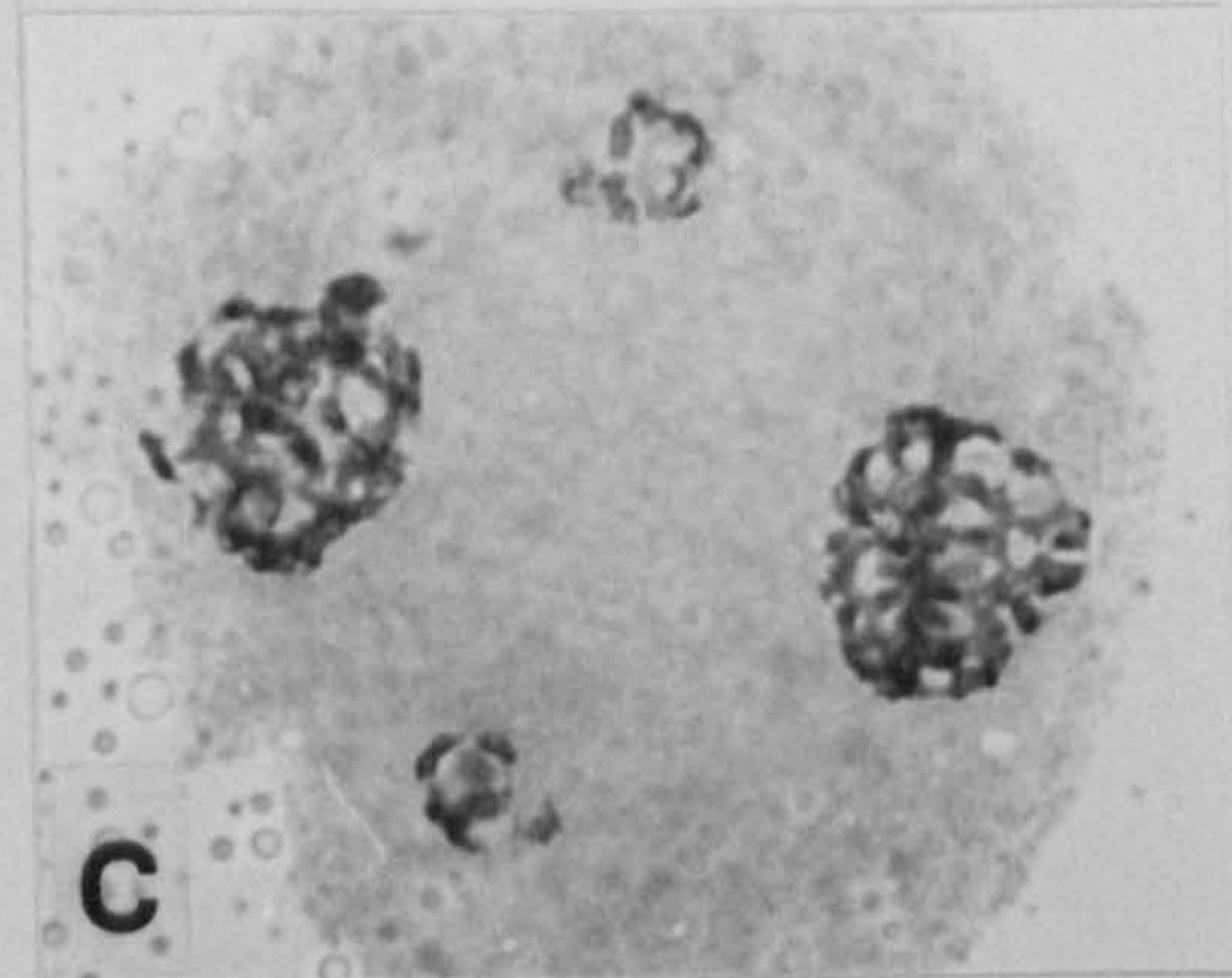
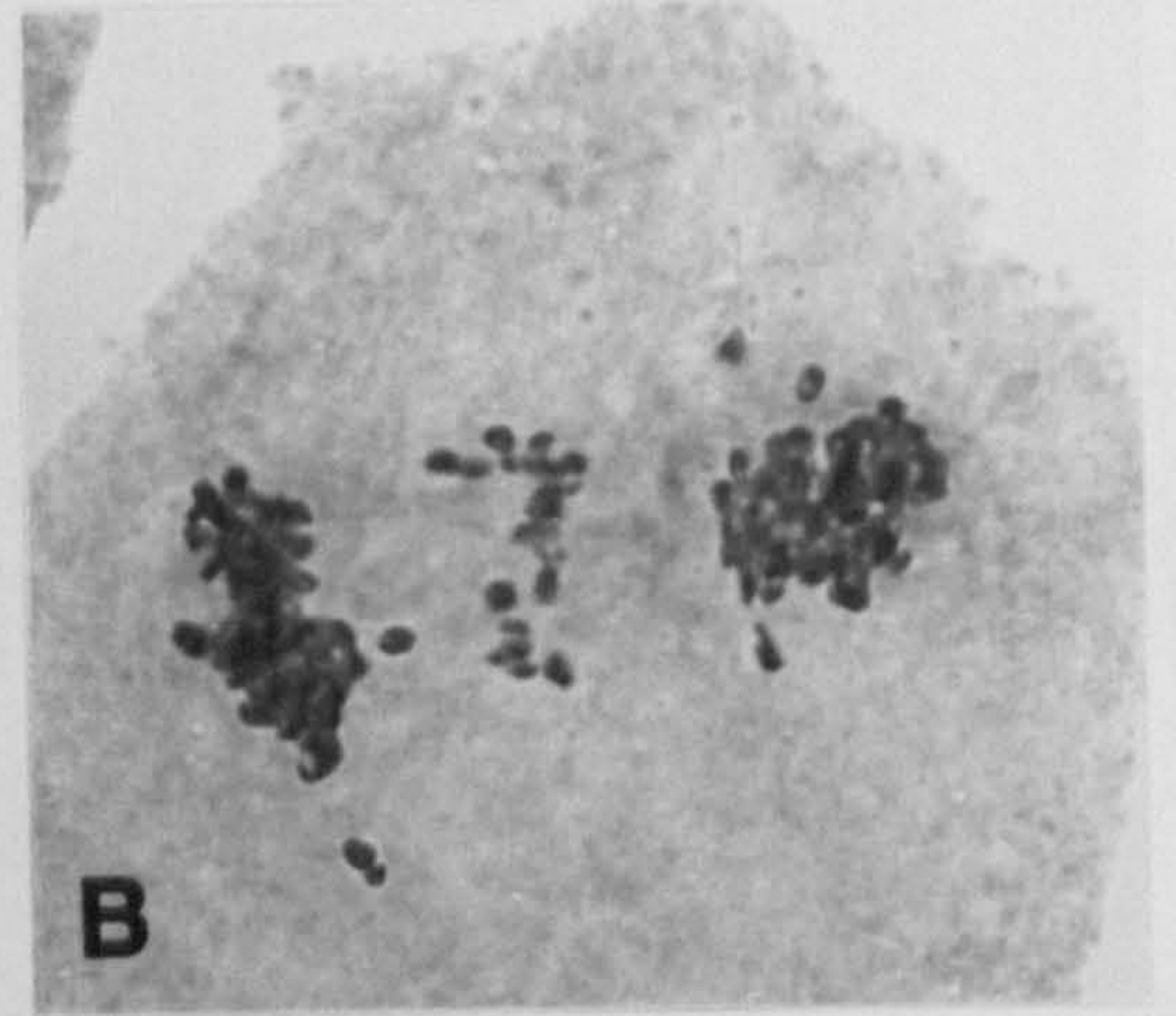
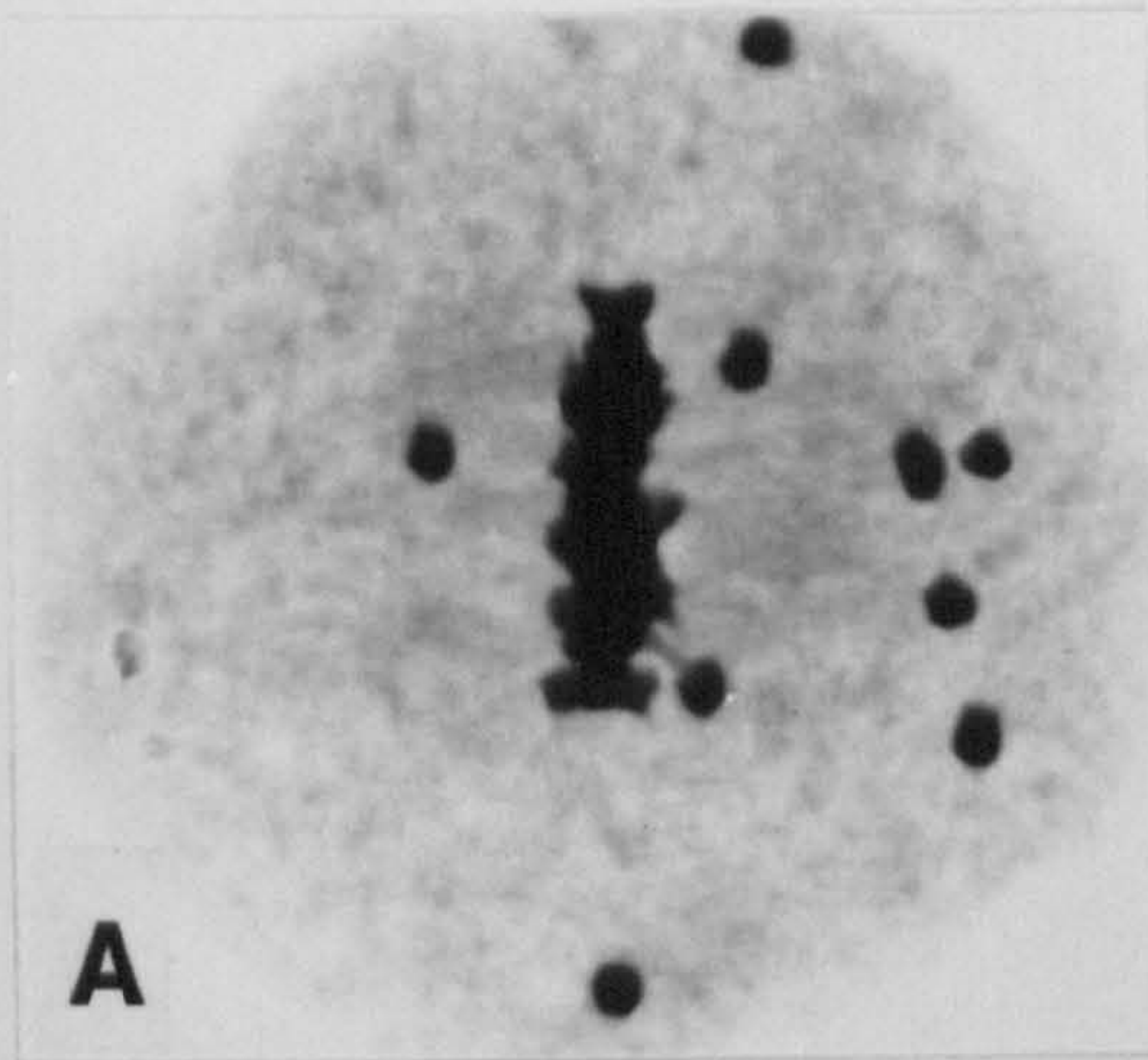


Plate 5.10 Meiosis in *R.japonica* x *F. baldschuanica* hybrids $2n = 54$

- | | | | |
|----------|-------------------------------------|----------|---|
| A | P80d with 10 univalents | B | P80b late anaphase with laggards |
| C | P80b telophase I with 2 micronuclei | D | P83b Telophase II with extraneous chromosomes |
| E | P80d telophase II with micronuclei | F | P80d Irregular tetrads (x500) |

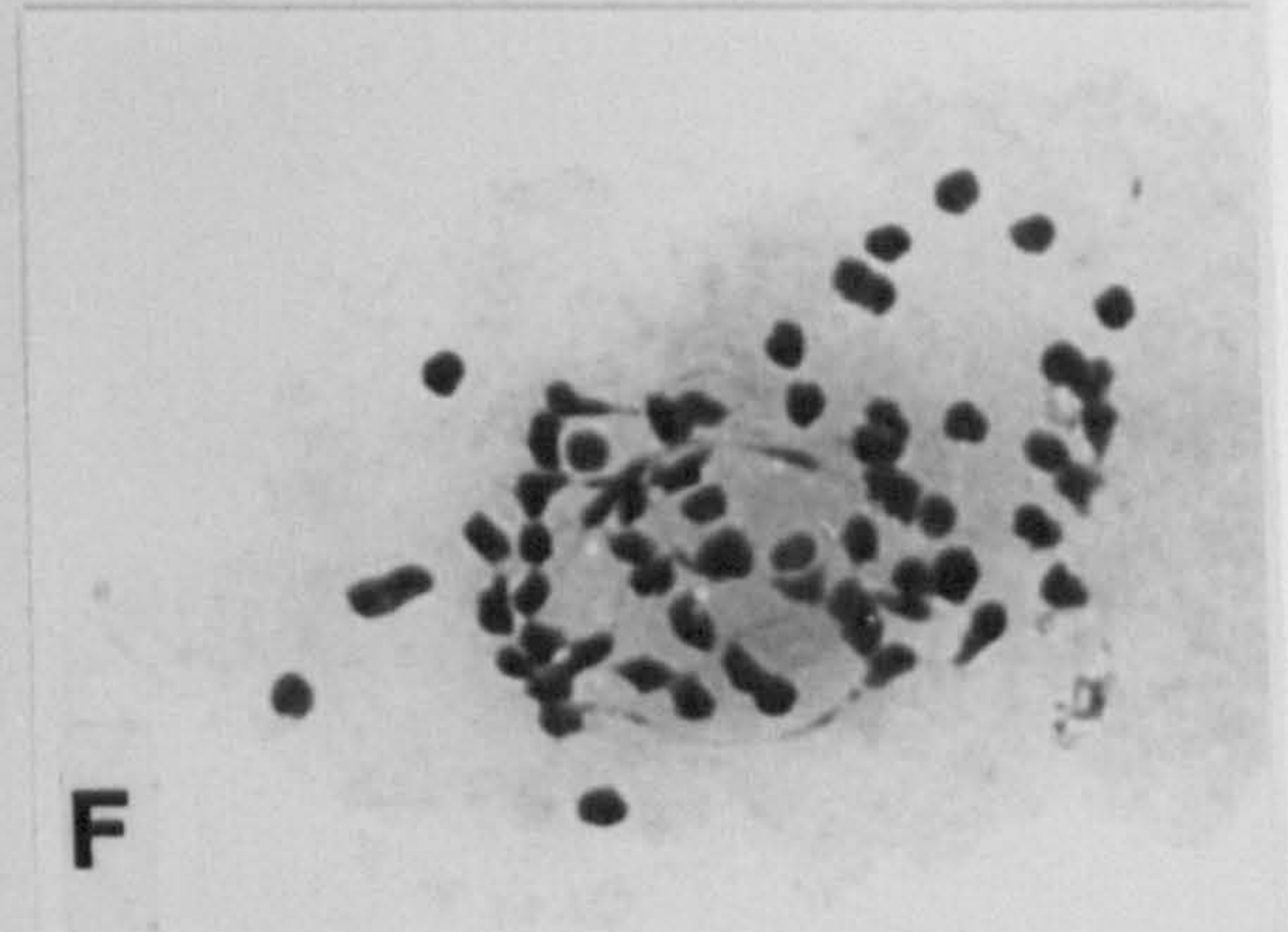
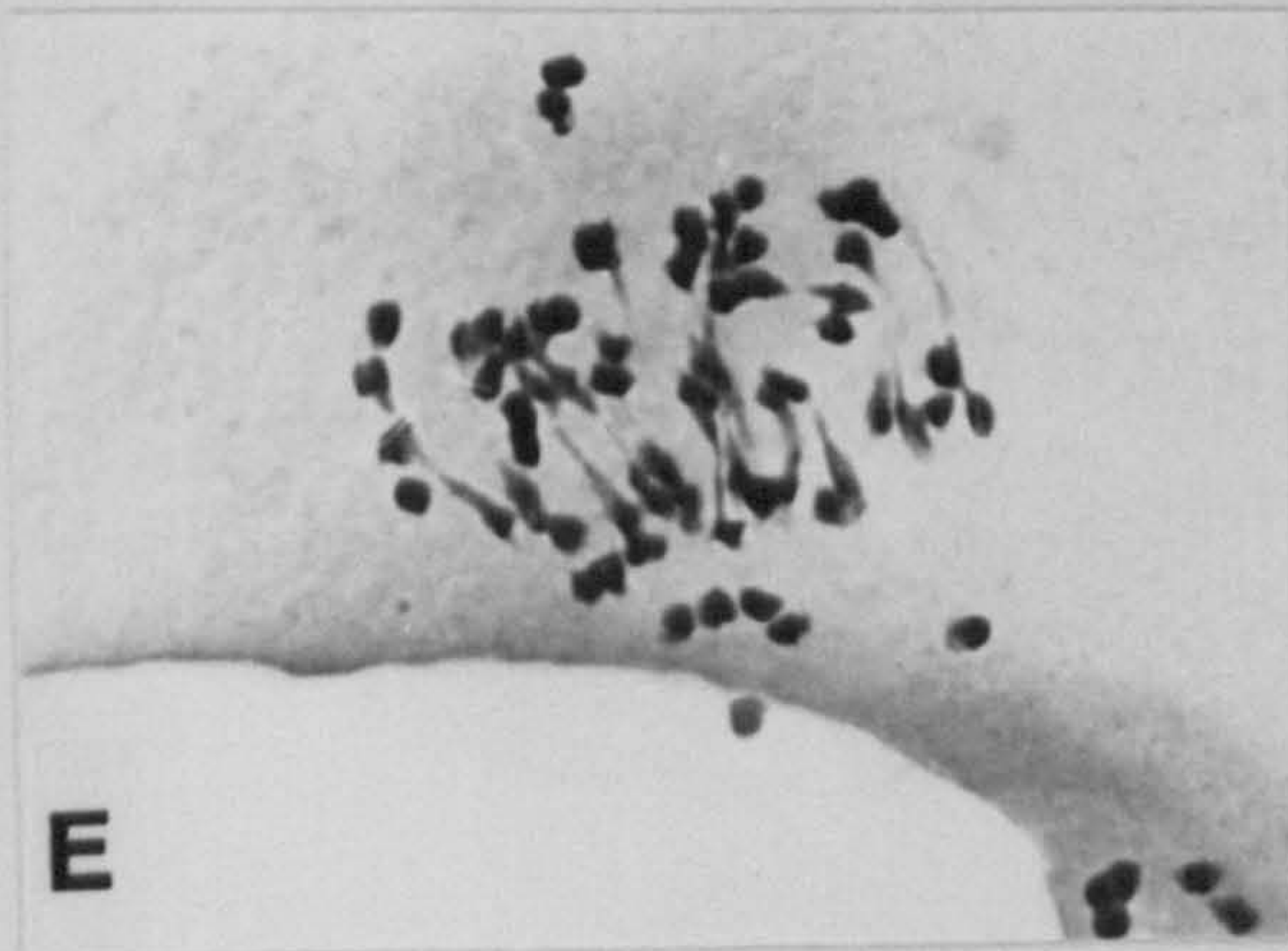
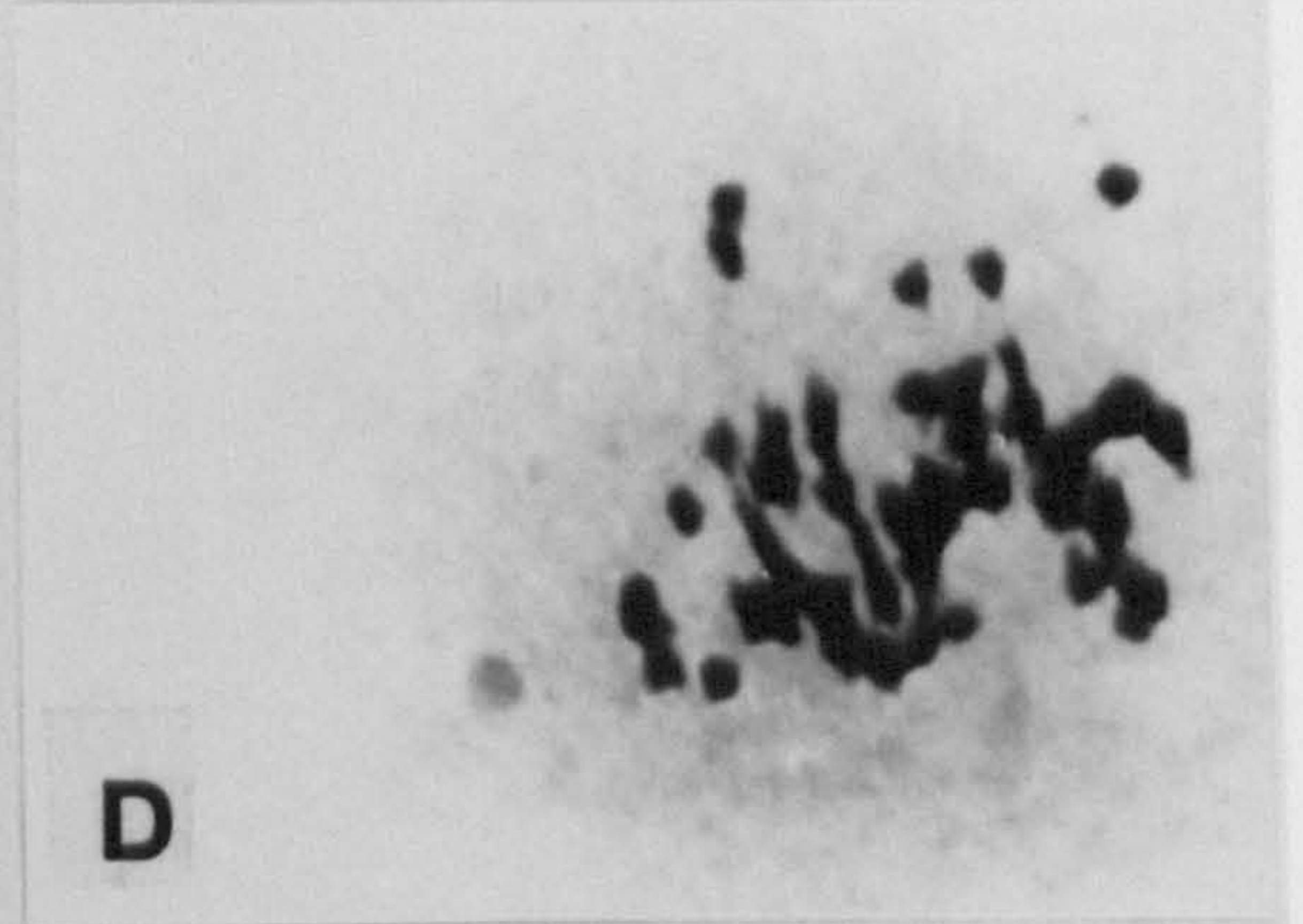
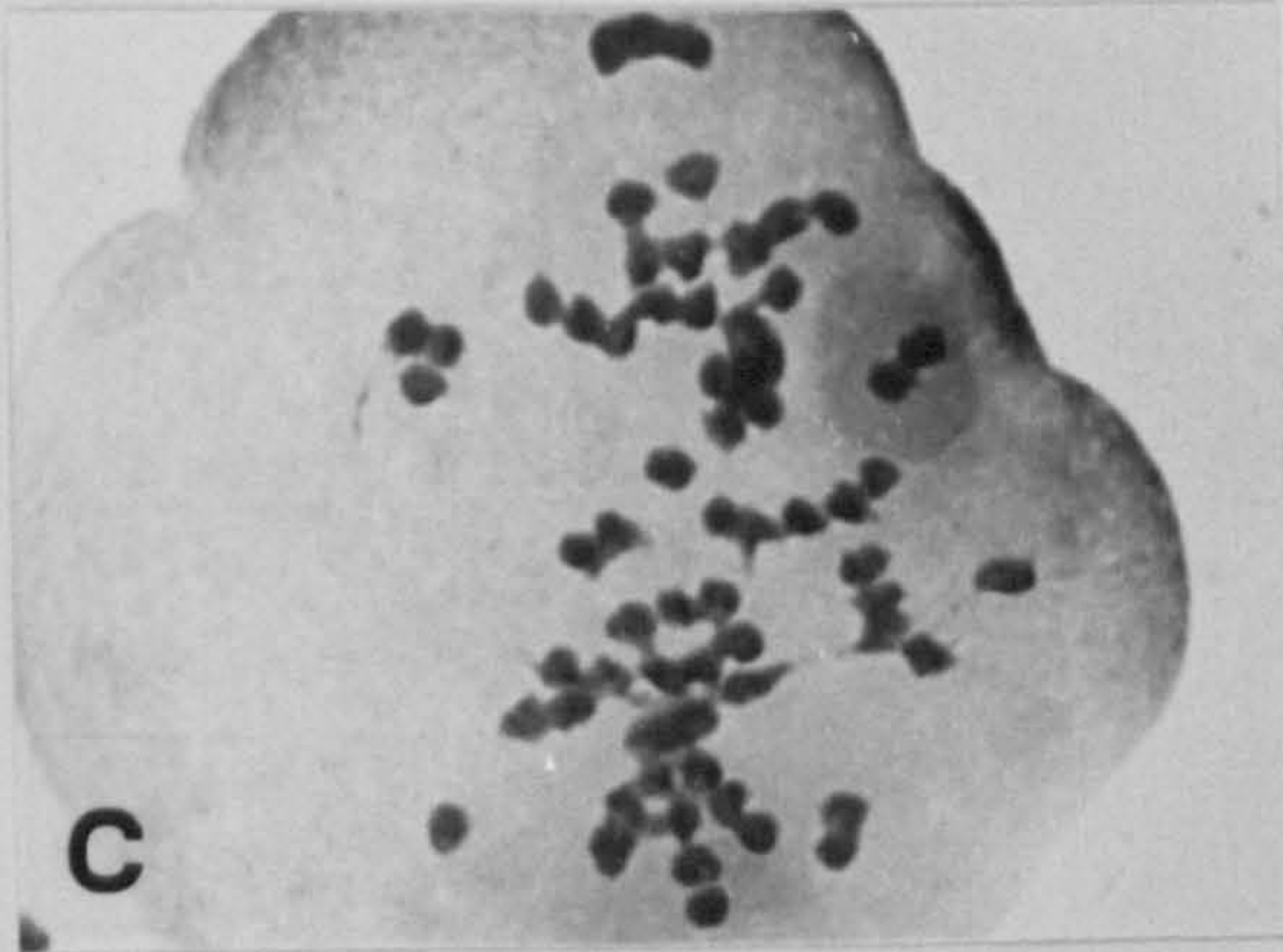
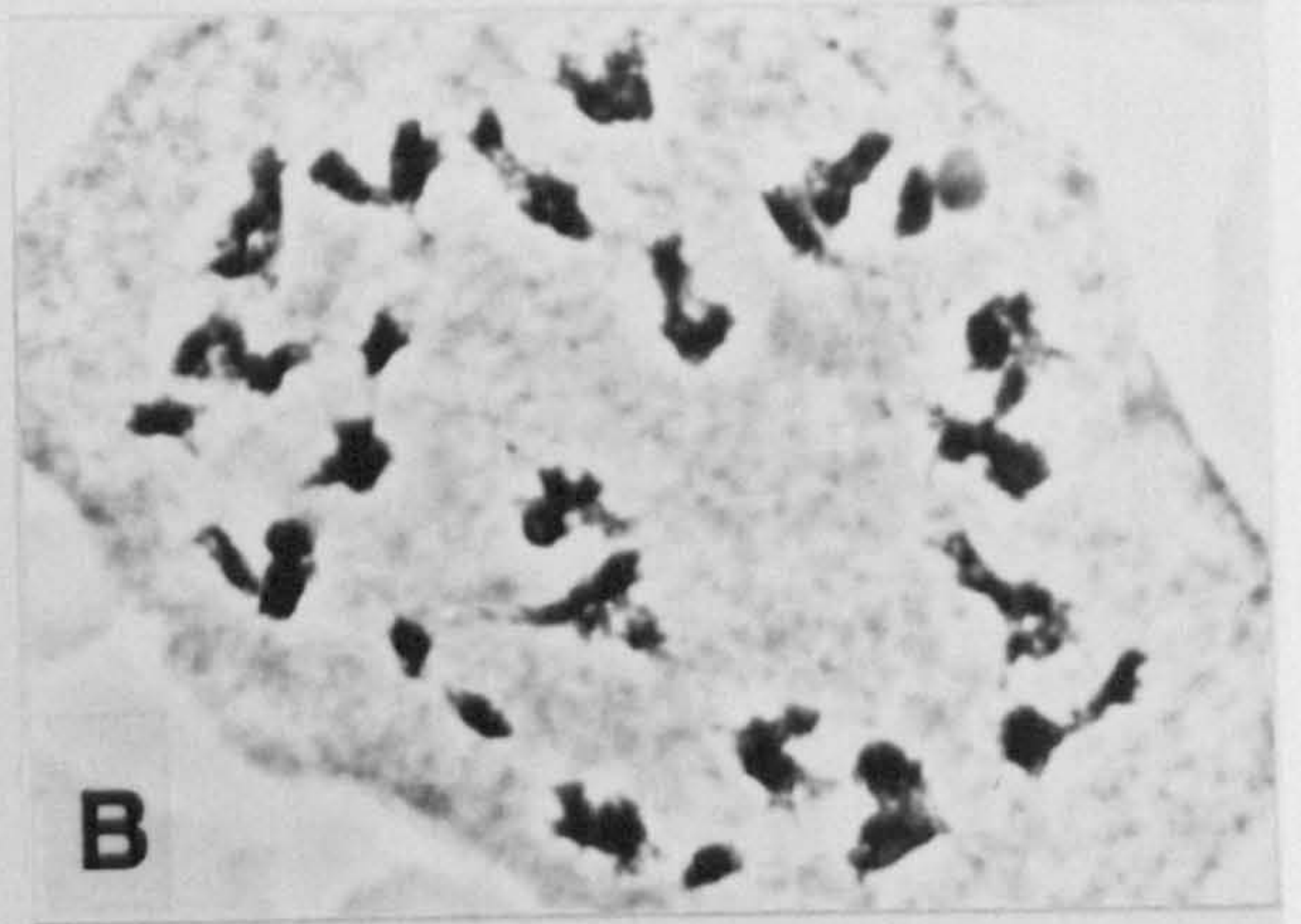
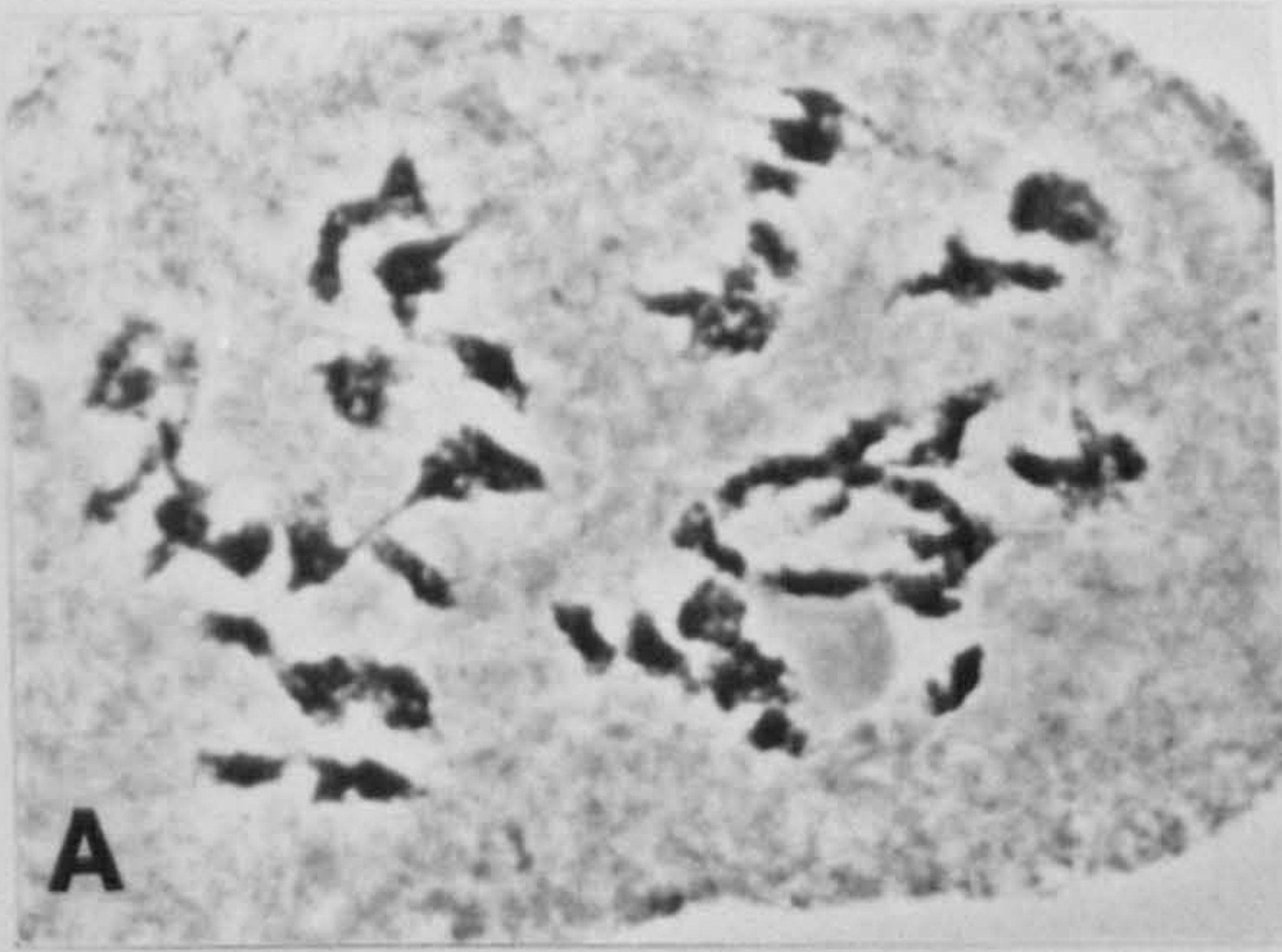


Plate 5.11 Meiosis in artificial *R.japonica* x *R.japonica* var.*compacta* hybrids $2n = 66$

A P76 10I 9II 6III 4IV

B P76

C P76a 17I 17II 5III

D P76a

E P76a

F P76b 30I 18II

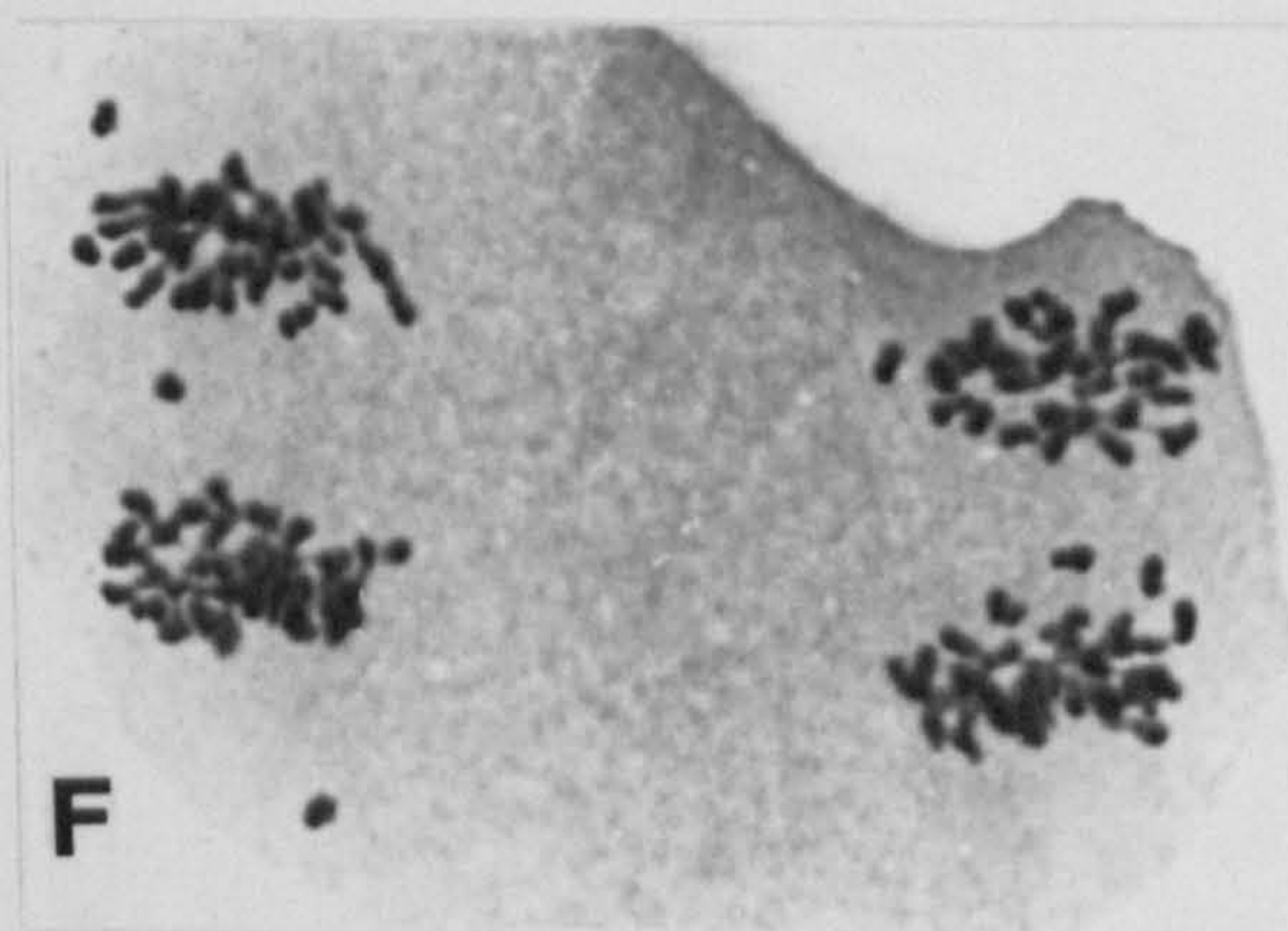
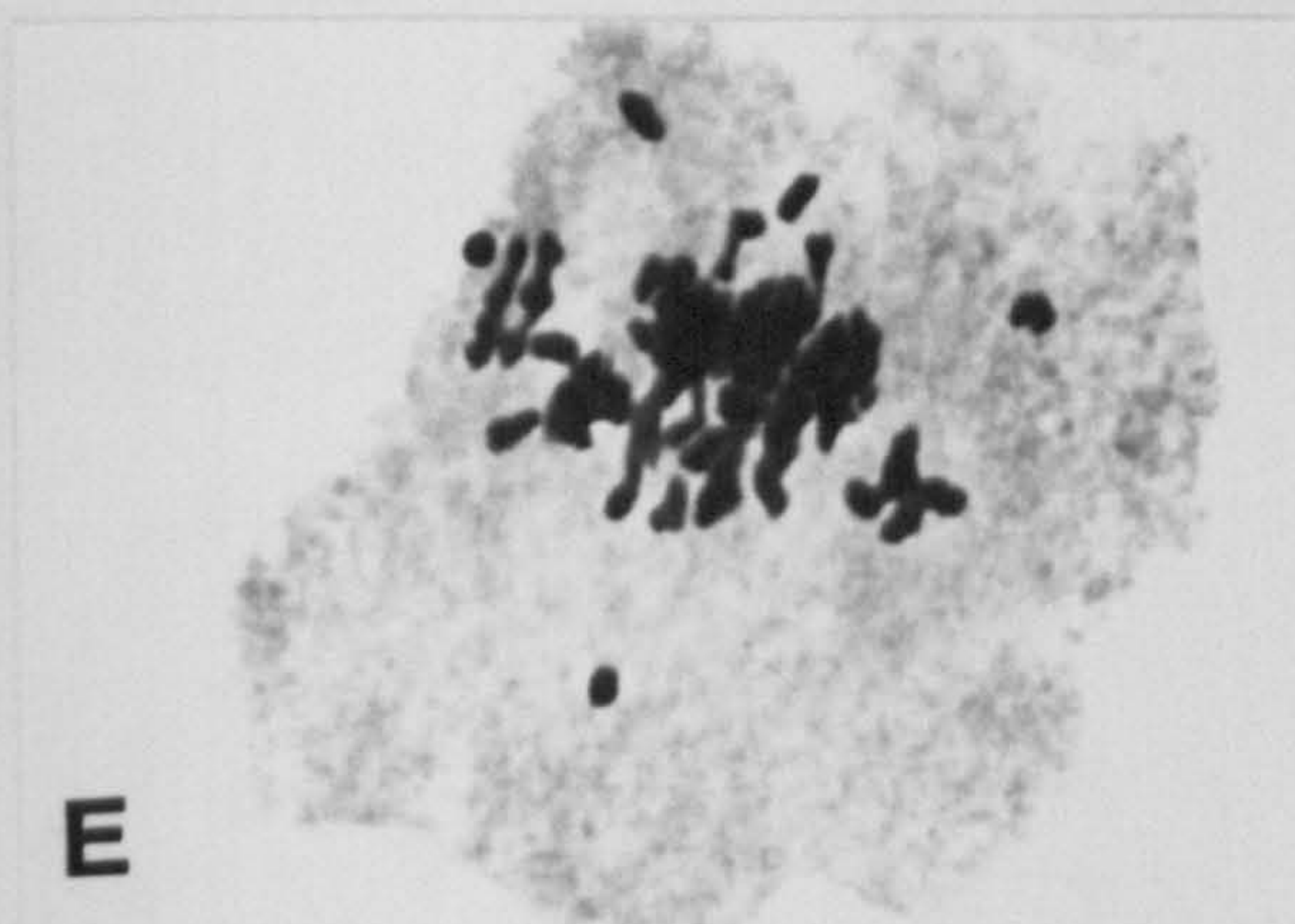
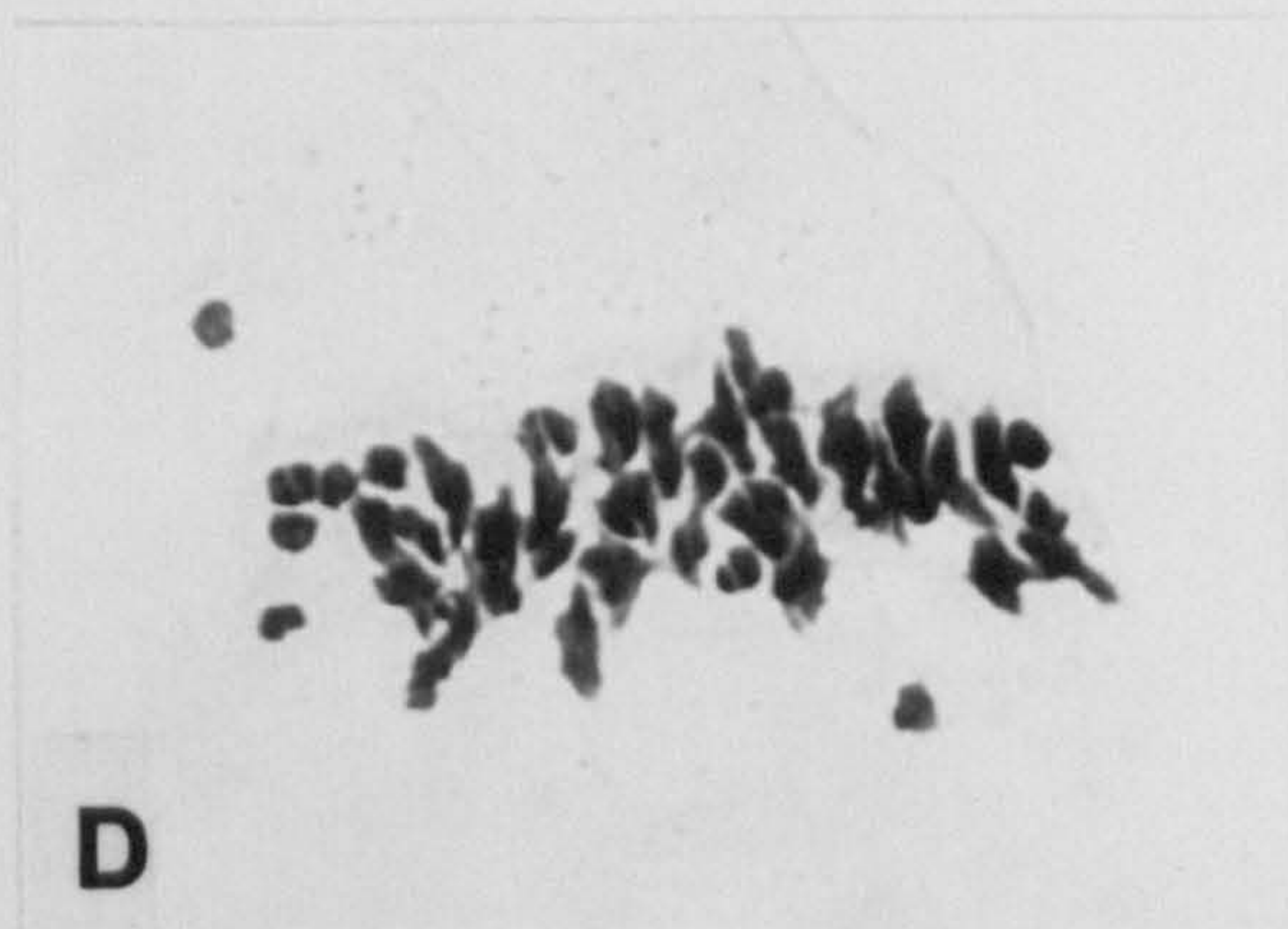
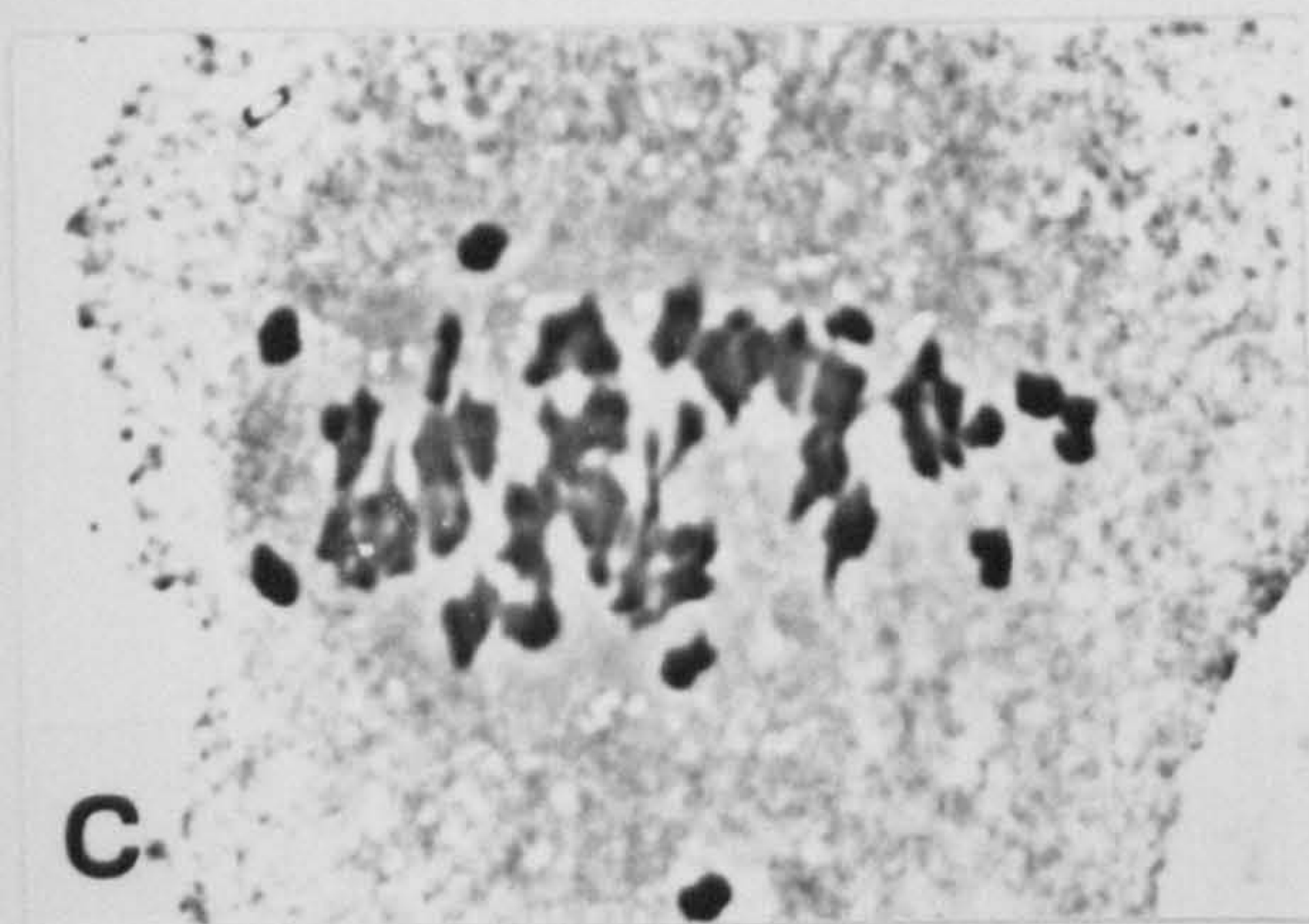
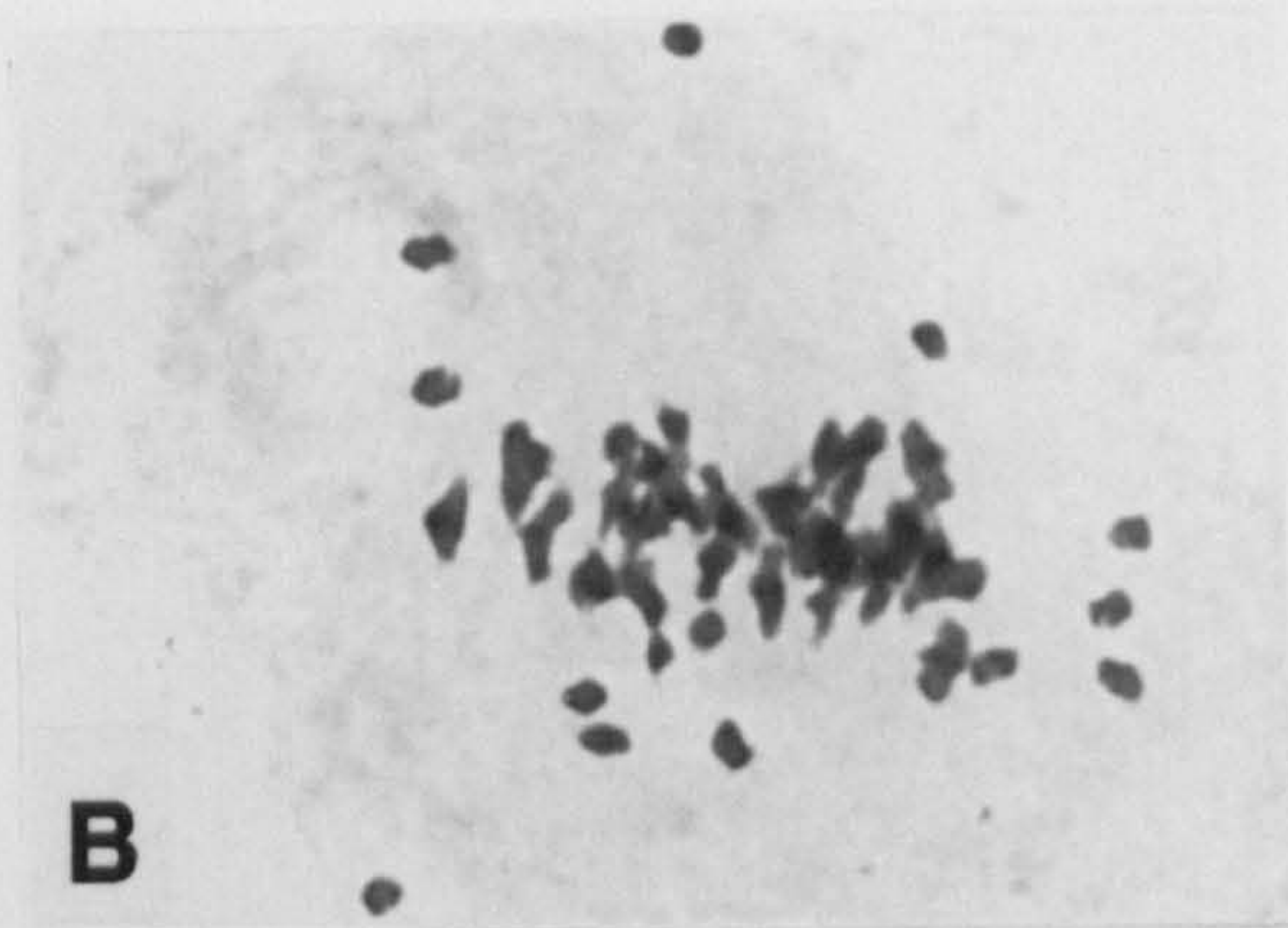
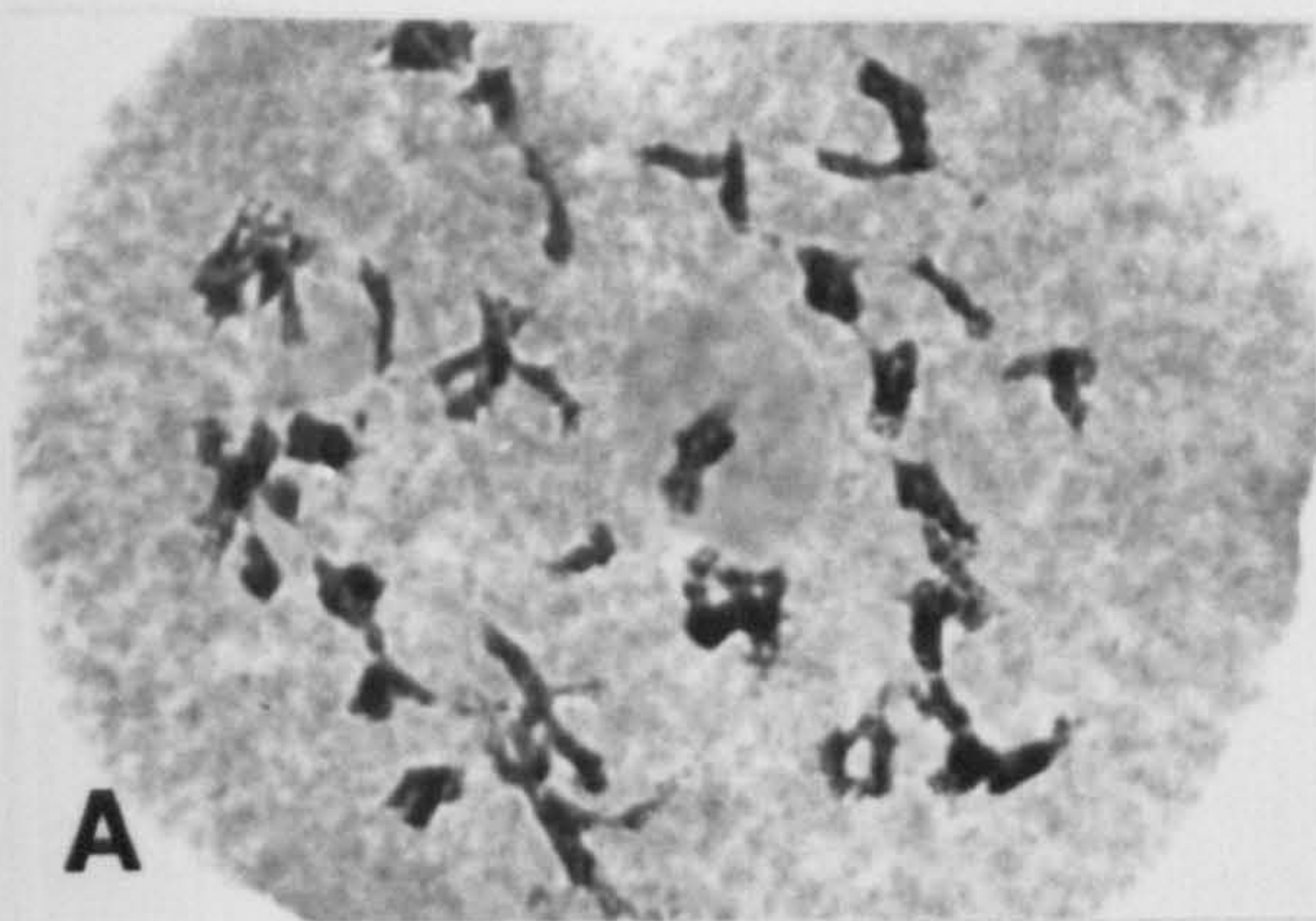


Plate 5.12 Meiosis in an artificial *R.japonica* x *R.sachalinensis* hybrid P75d $2n = 66$

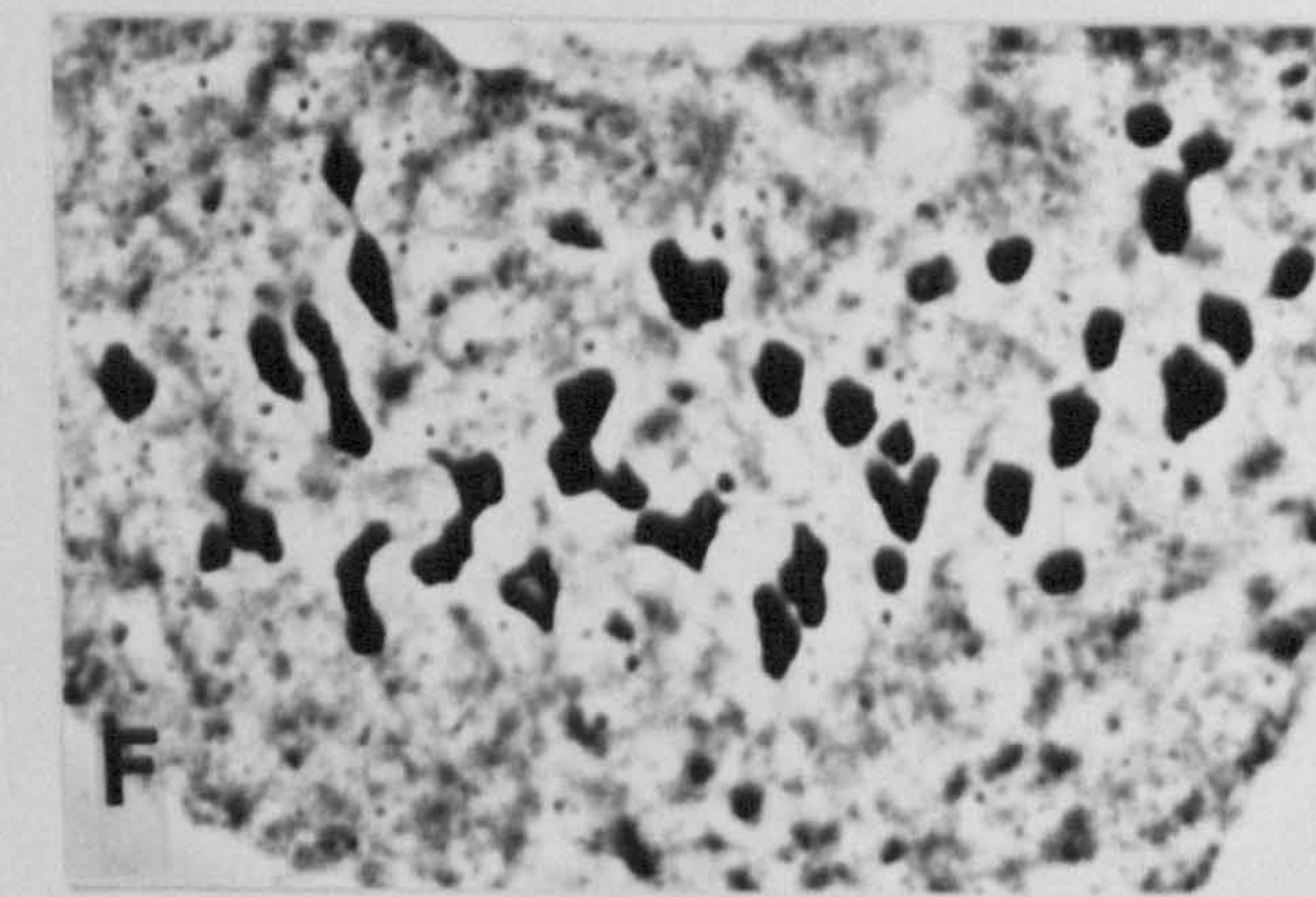
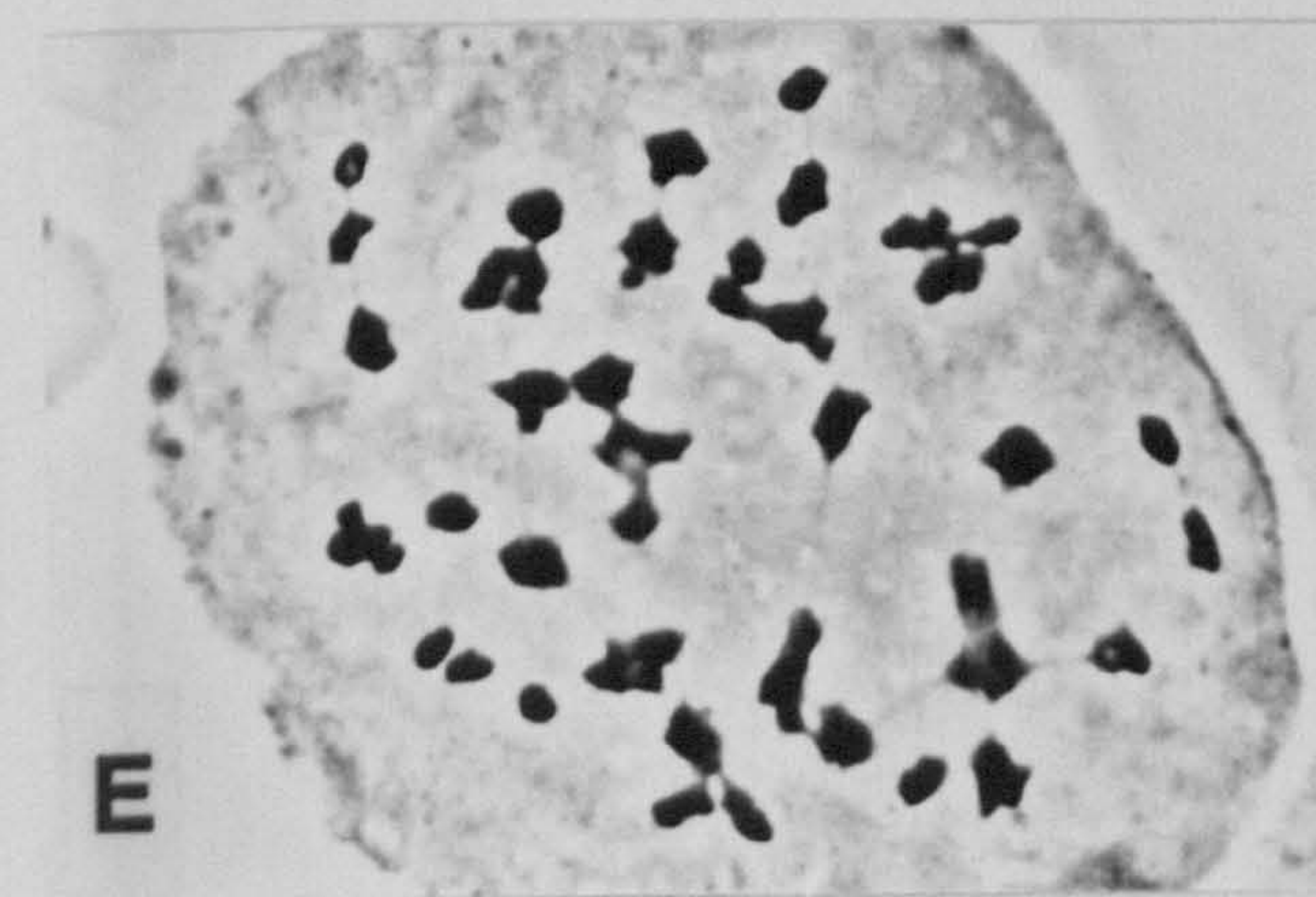
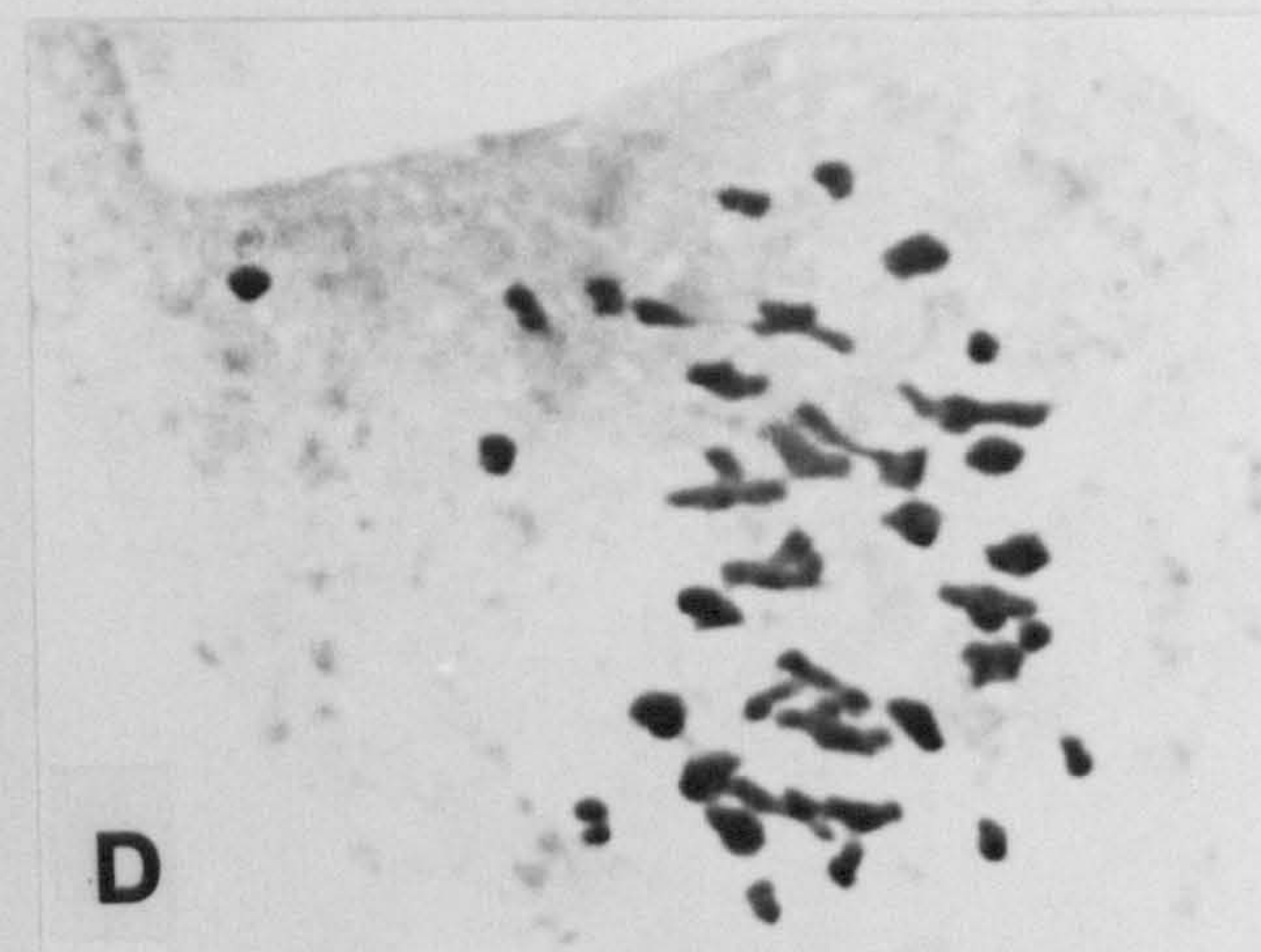
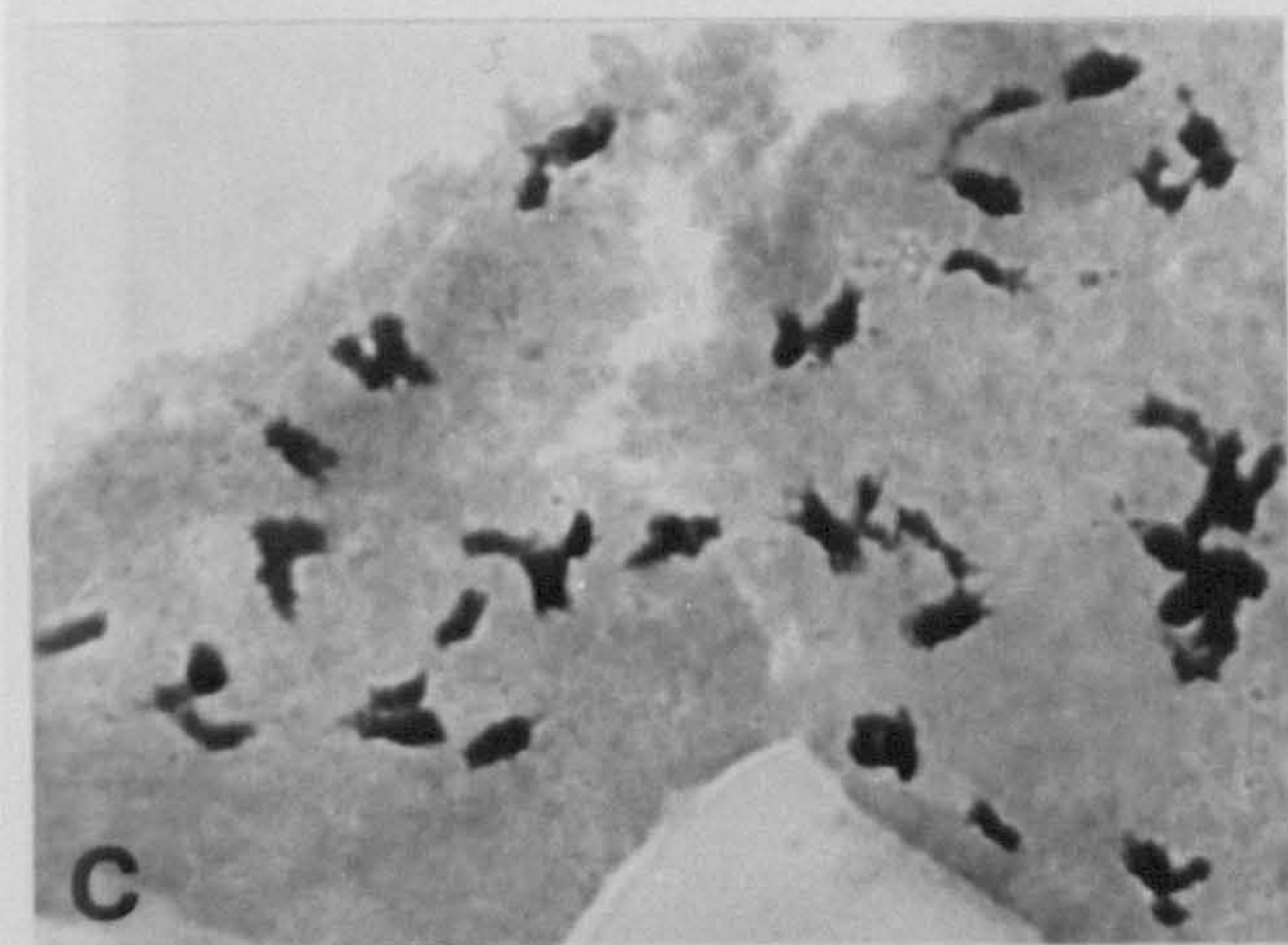
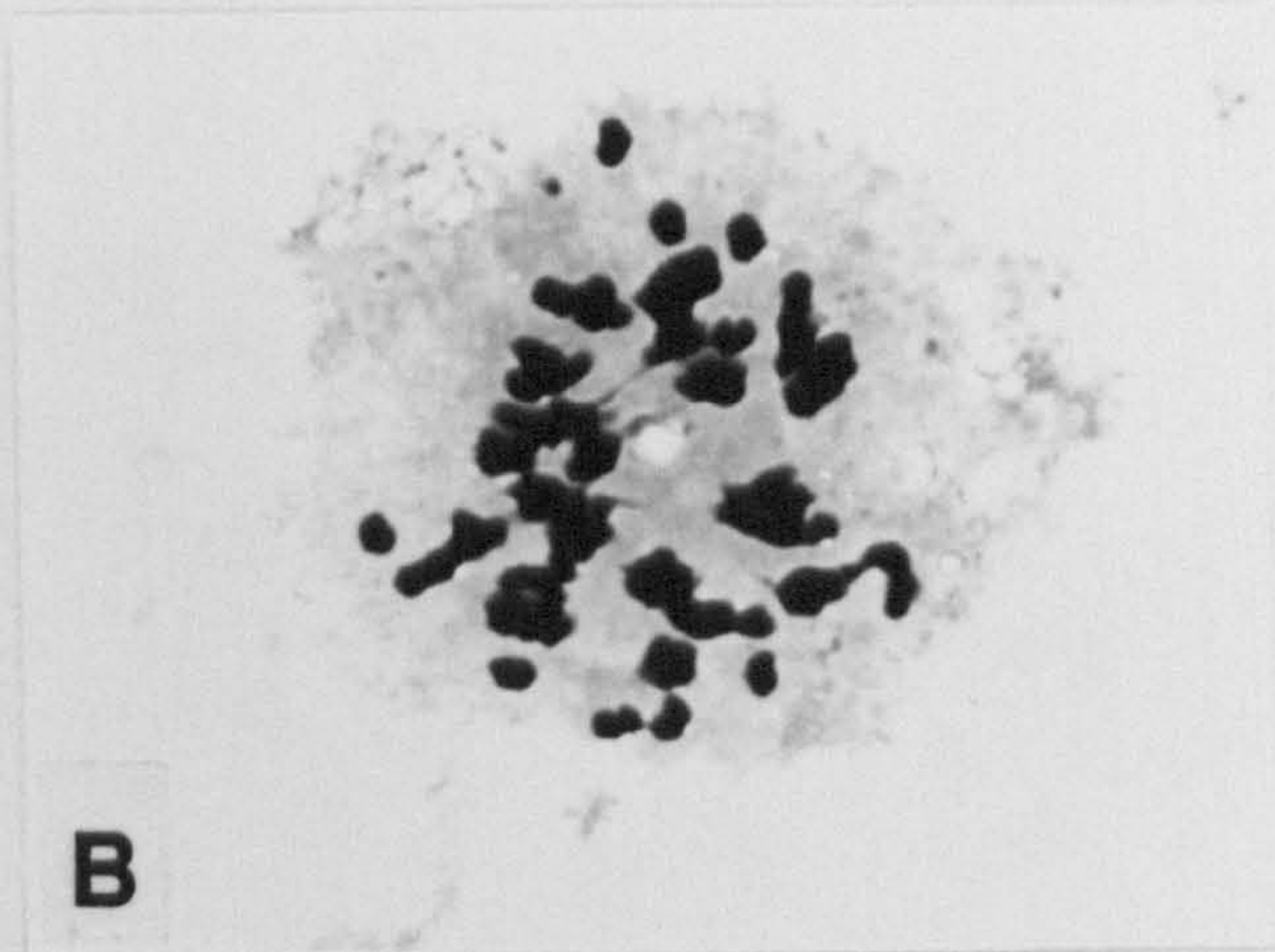
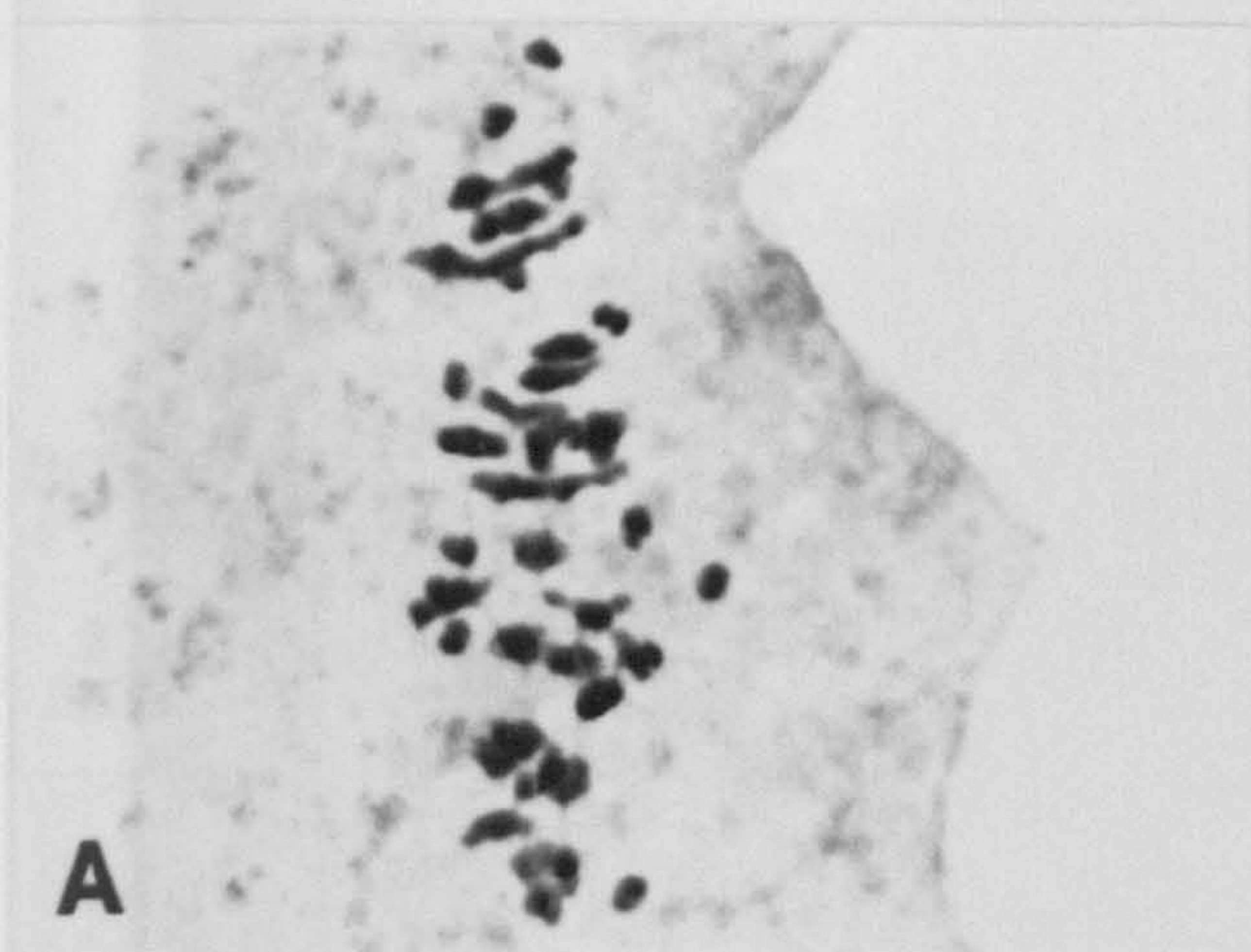


Plate 5.13 Meiosis in natural *R.japonica* x *R.sachalinensis* hybrids $2n = 66$

A P32 22I 13II 2III 2IV

B,C P119

D P32 18I 23II 1III

E P32 21I 22II

F P32 12I 18II 5III

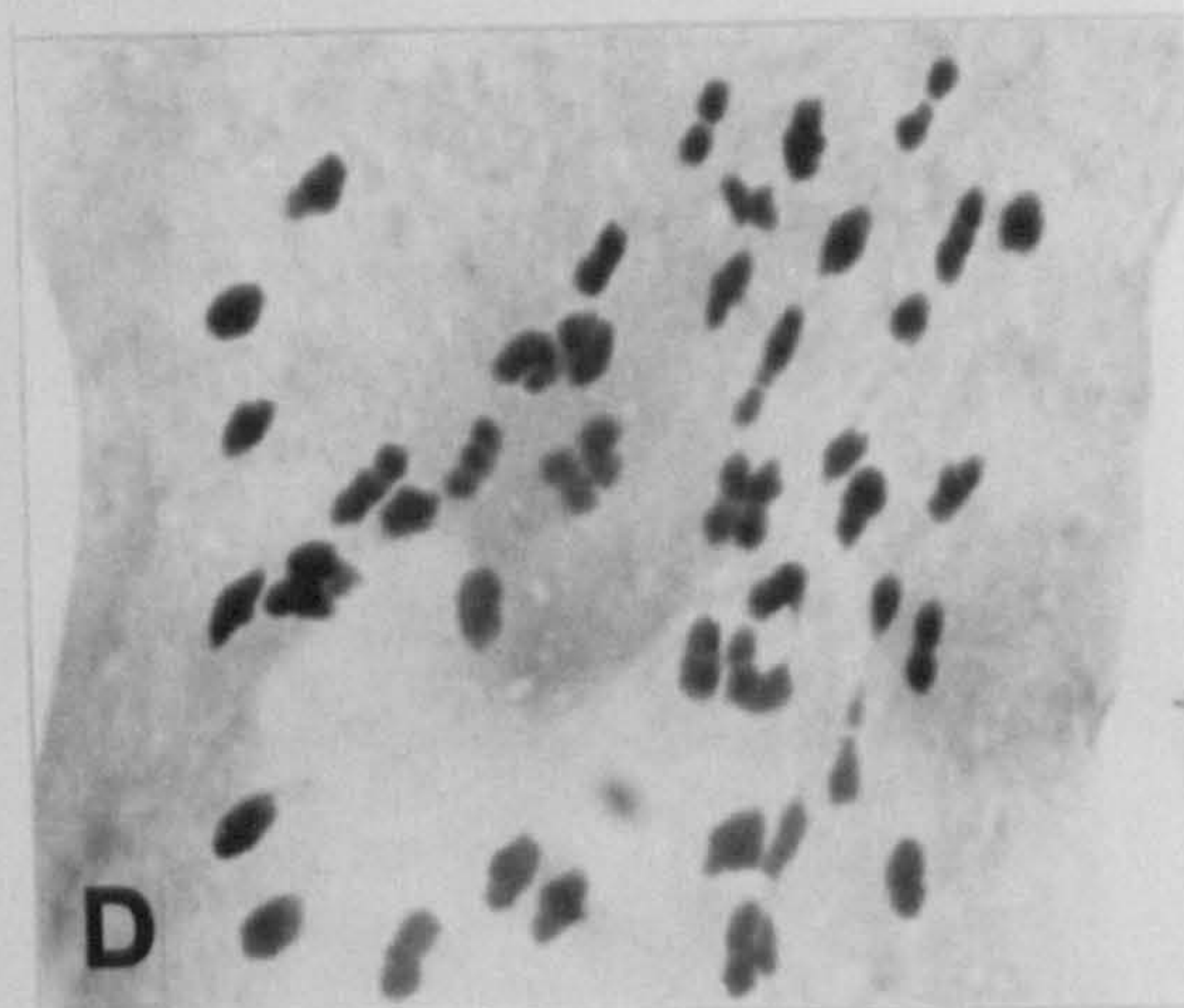
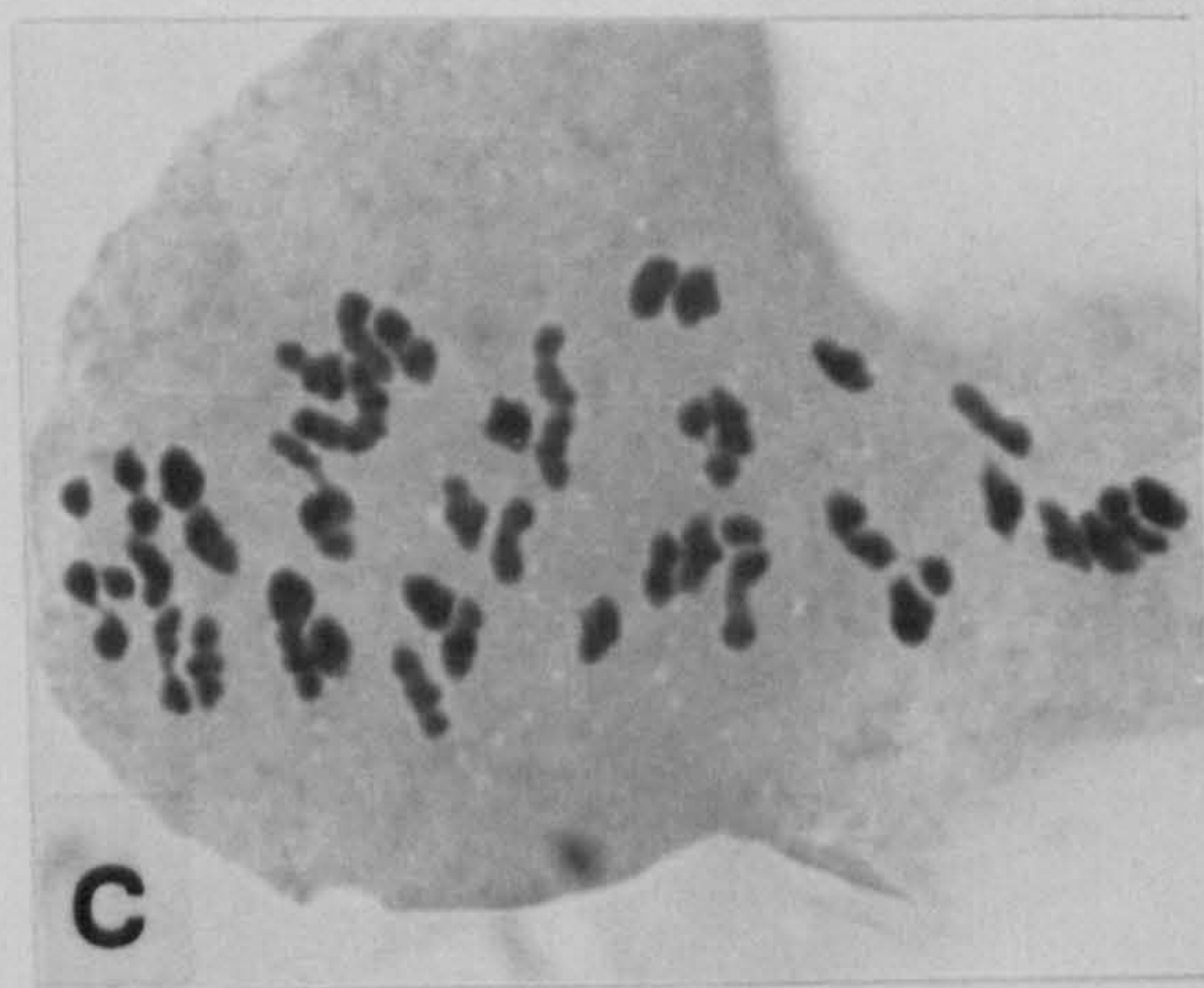
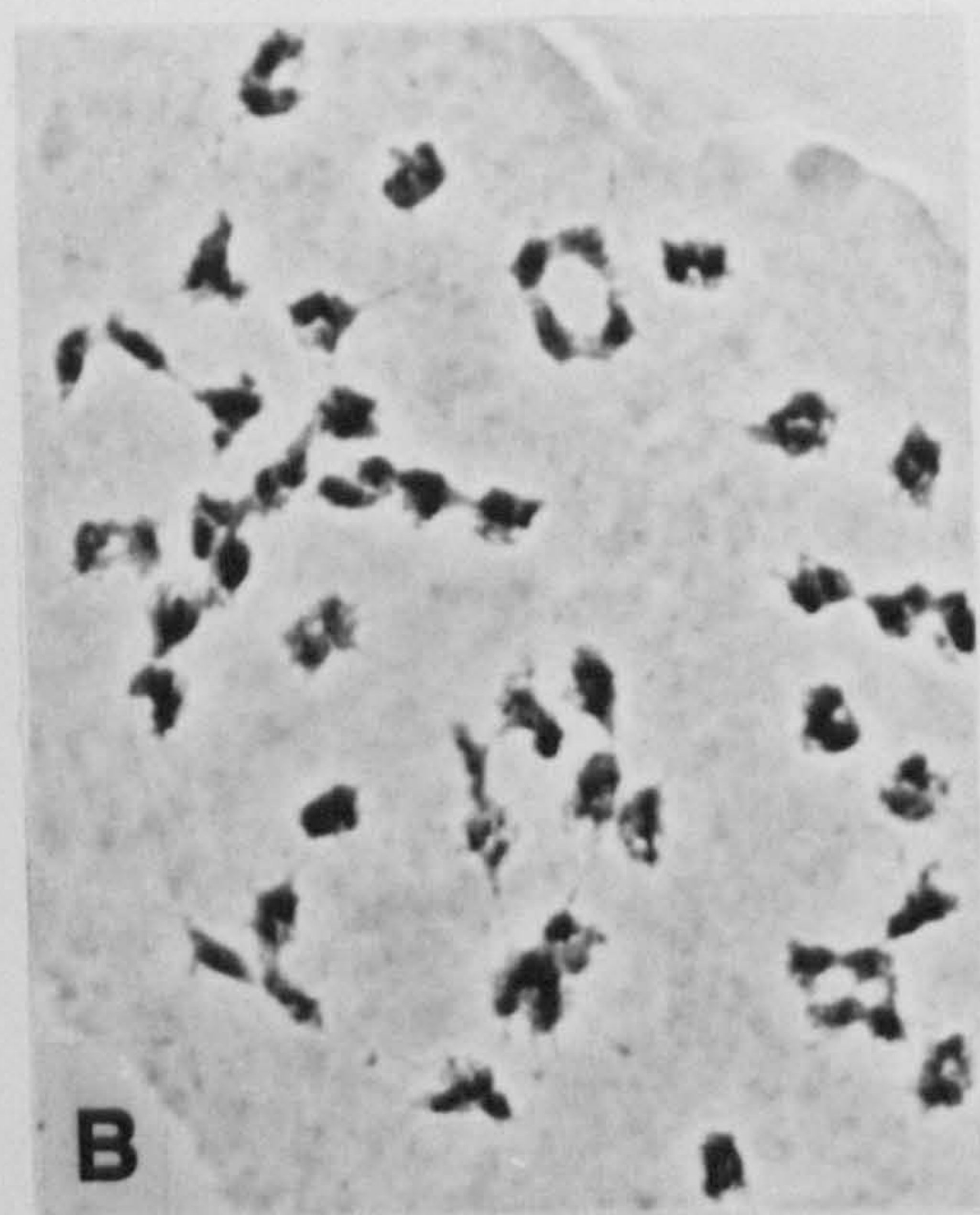
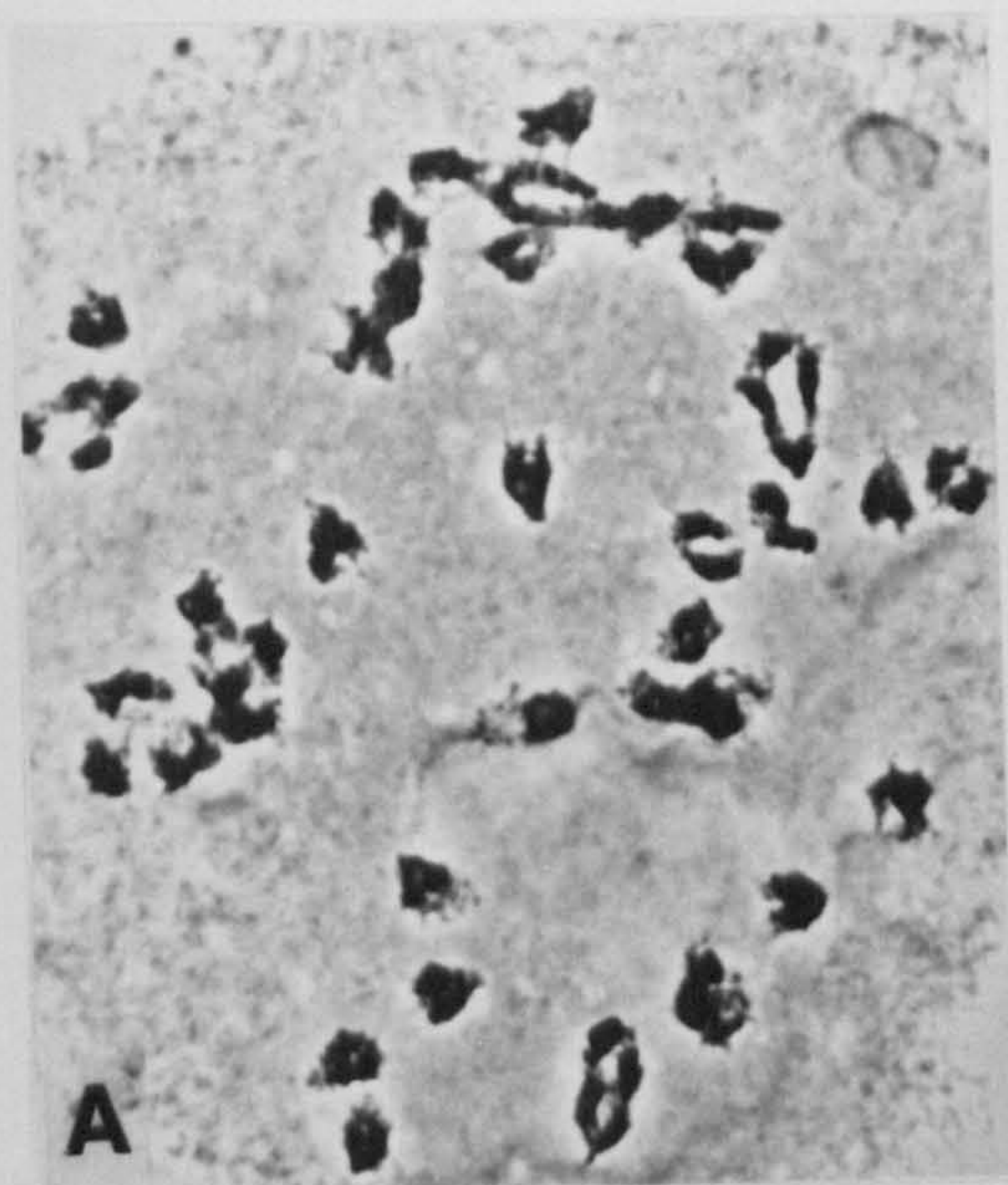


Plate 5.14.1 Octoploid hybrid meiosis

A - D putative *R.japonica* x *R. sachalinensis* P50 $2n = 88$

A 31II 5IV 1VI

B 40II 2IV

C 2I 40II 1IV

D 36II 4IV

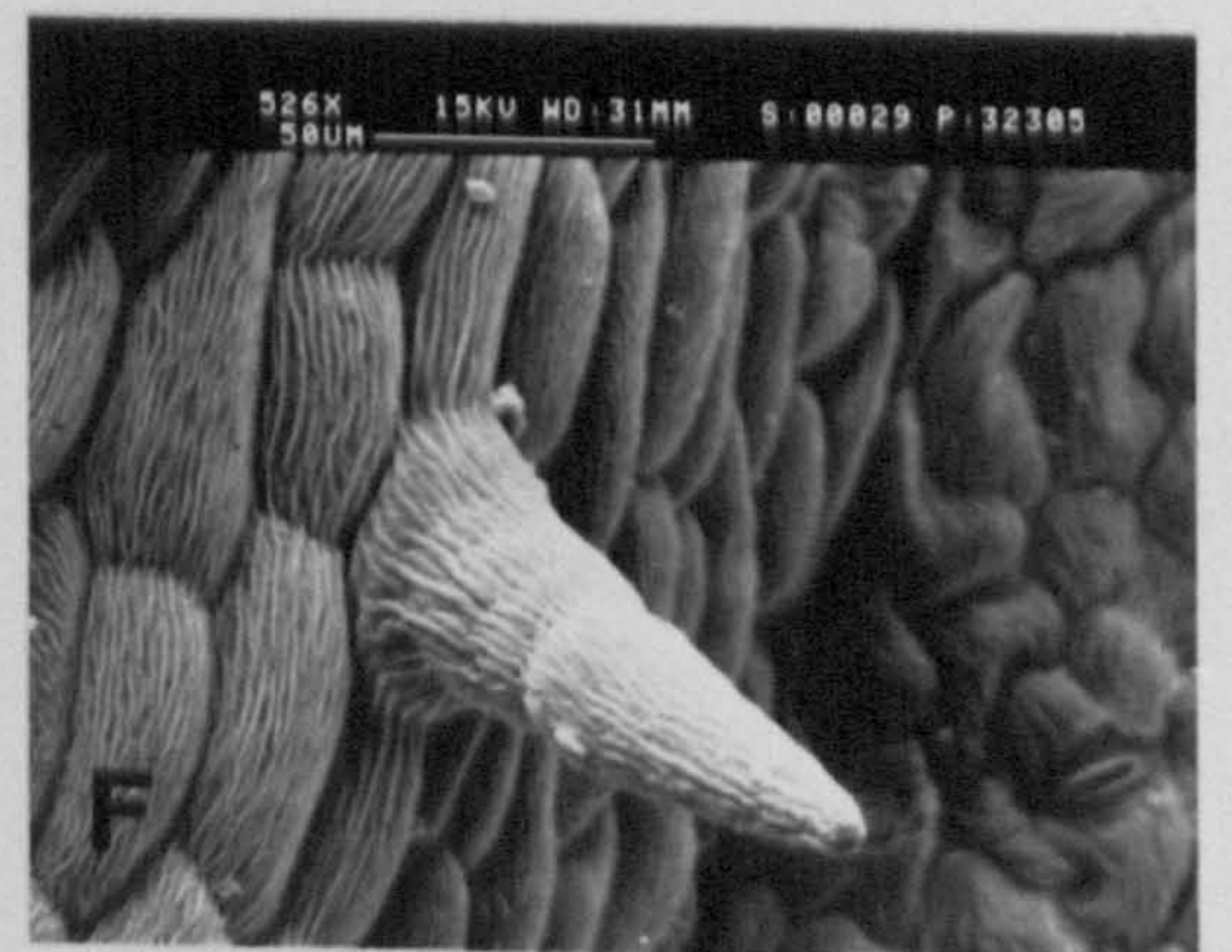
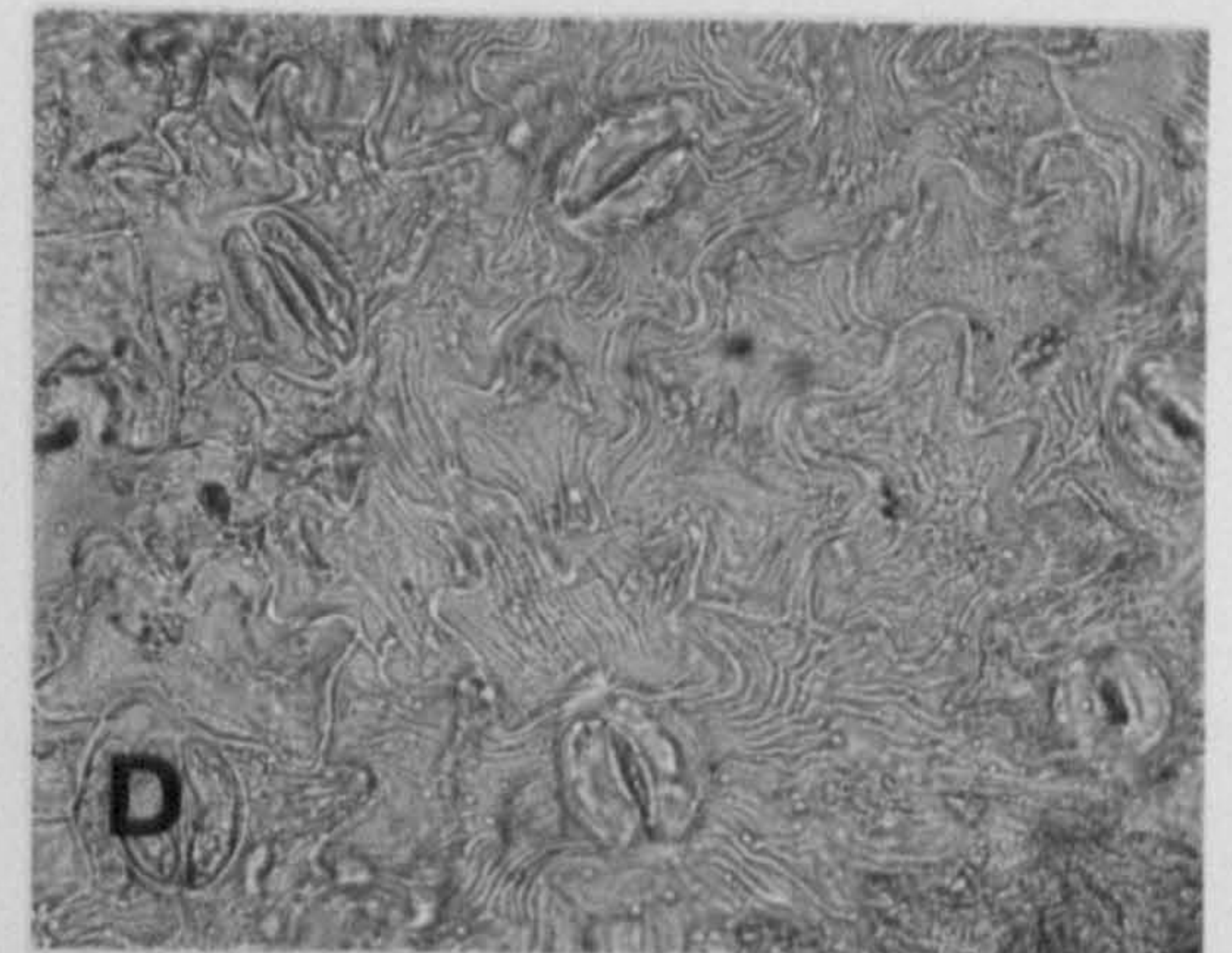
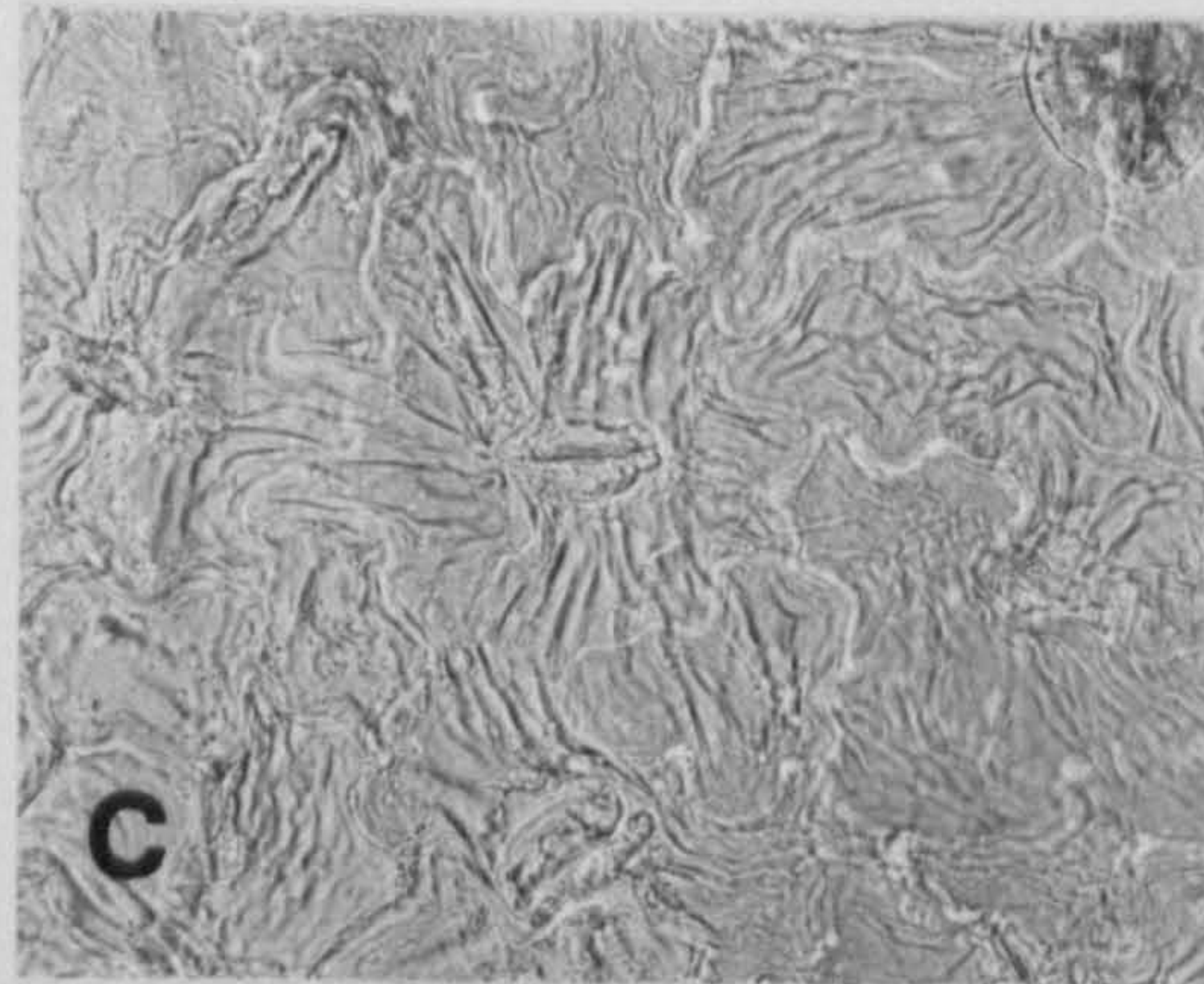
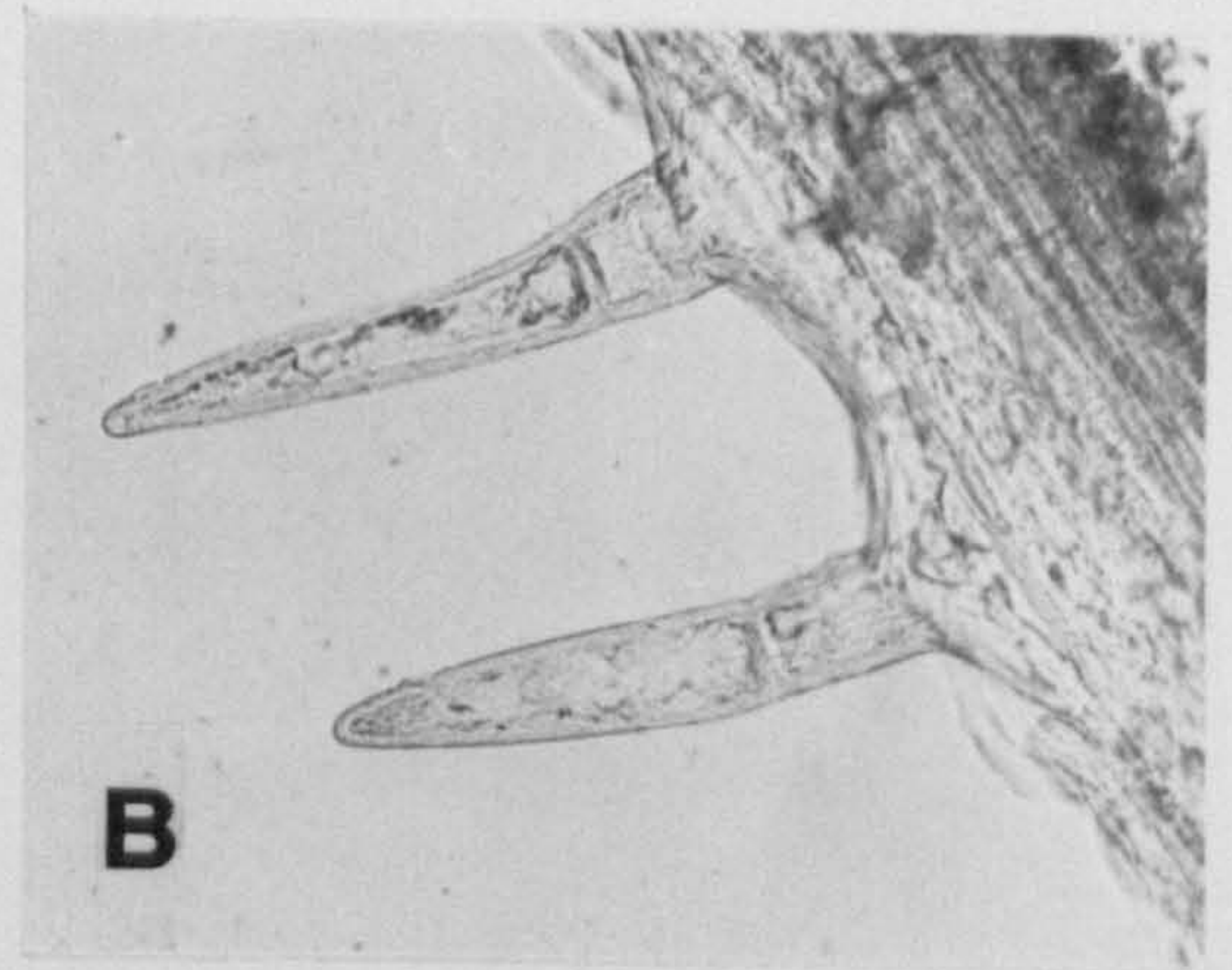
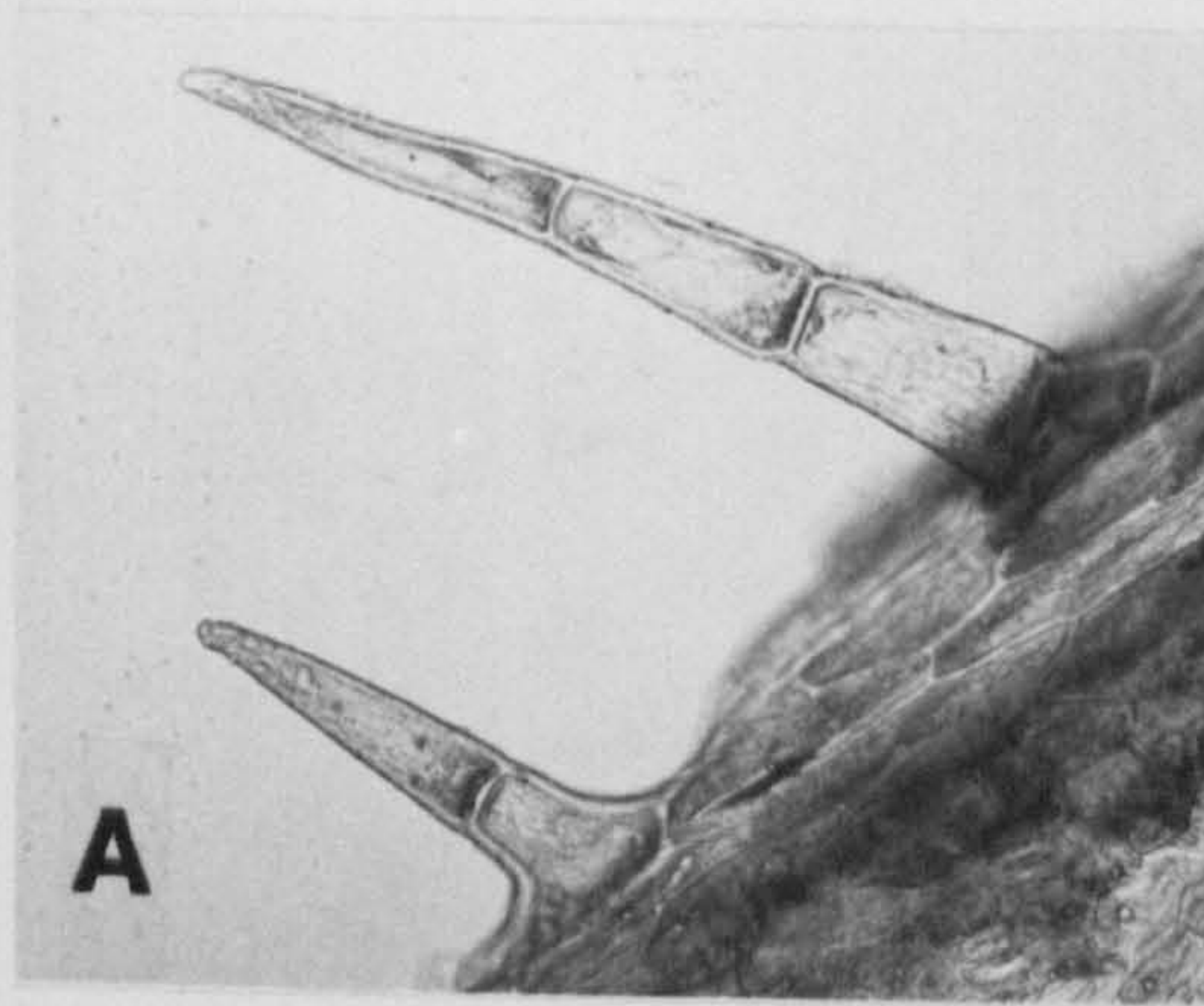


Plate 5. 14.2R. *sachalinensis* x *F. baldschuanica*

A,D Trichomes and epidermis P104a

B,C Trichomes and epidermis of artificial hybrid P101b

E,F SEM of epidermis and trichomes of P140a

NB all trichomes X 160 and epidermal preparations X 312 unless otherwise indicated

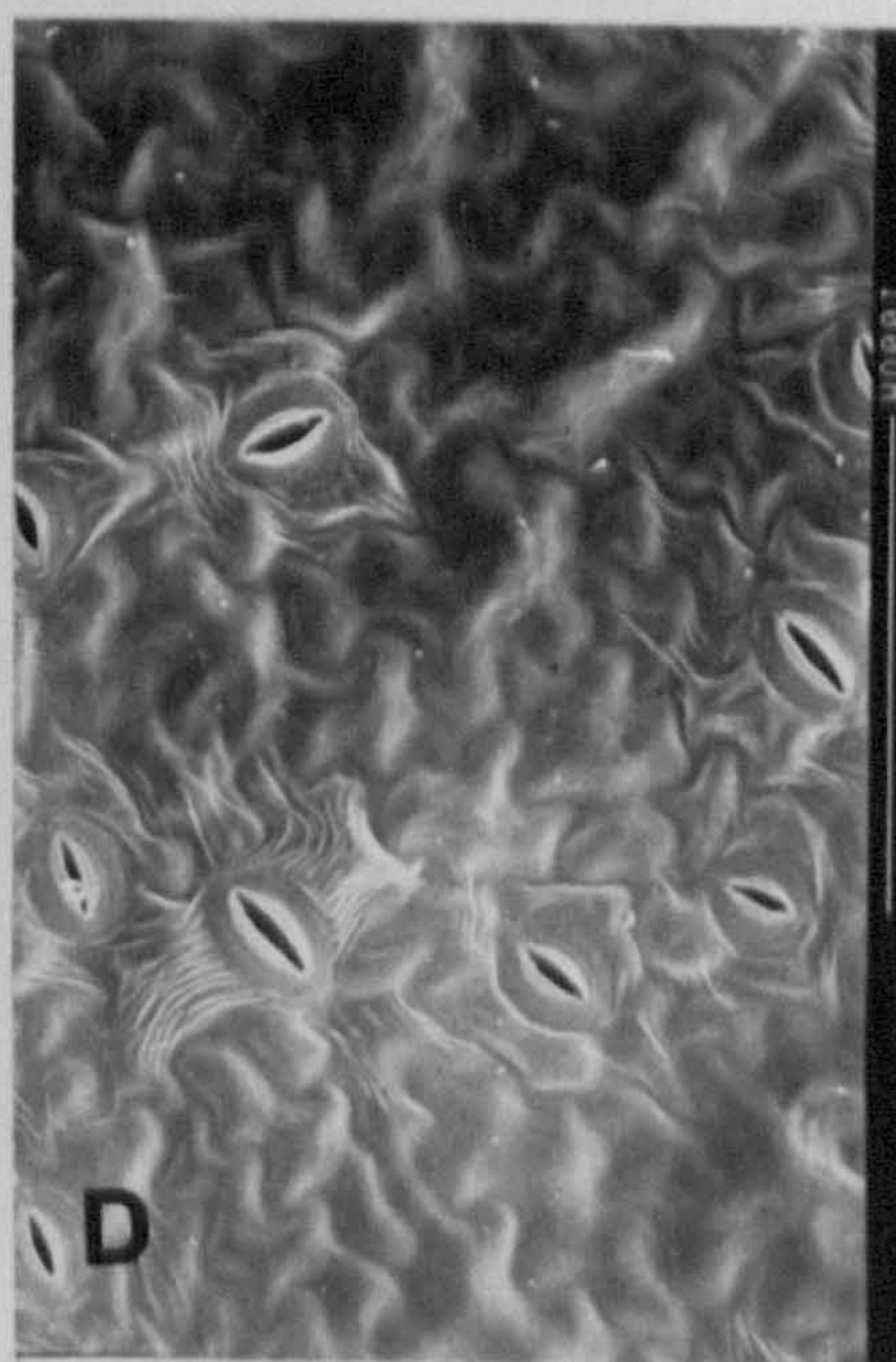
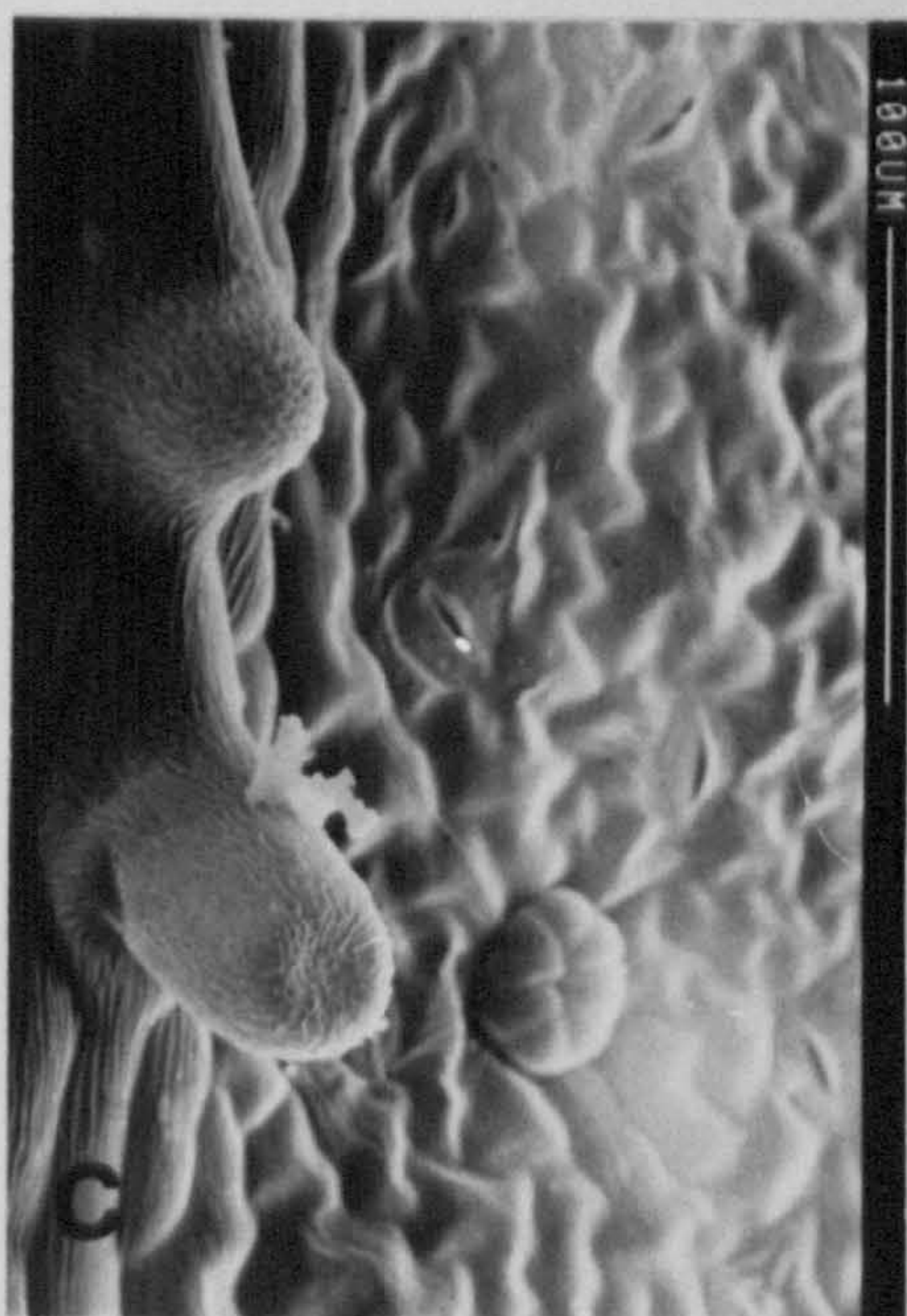
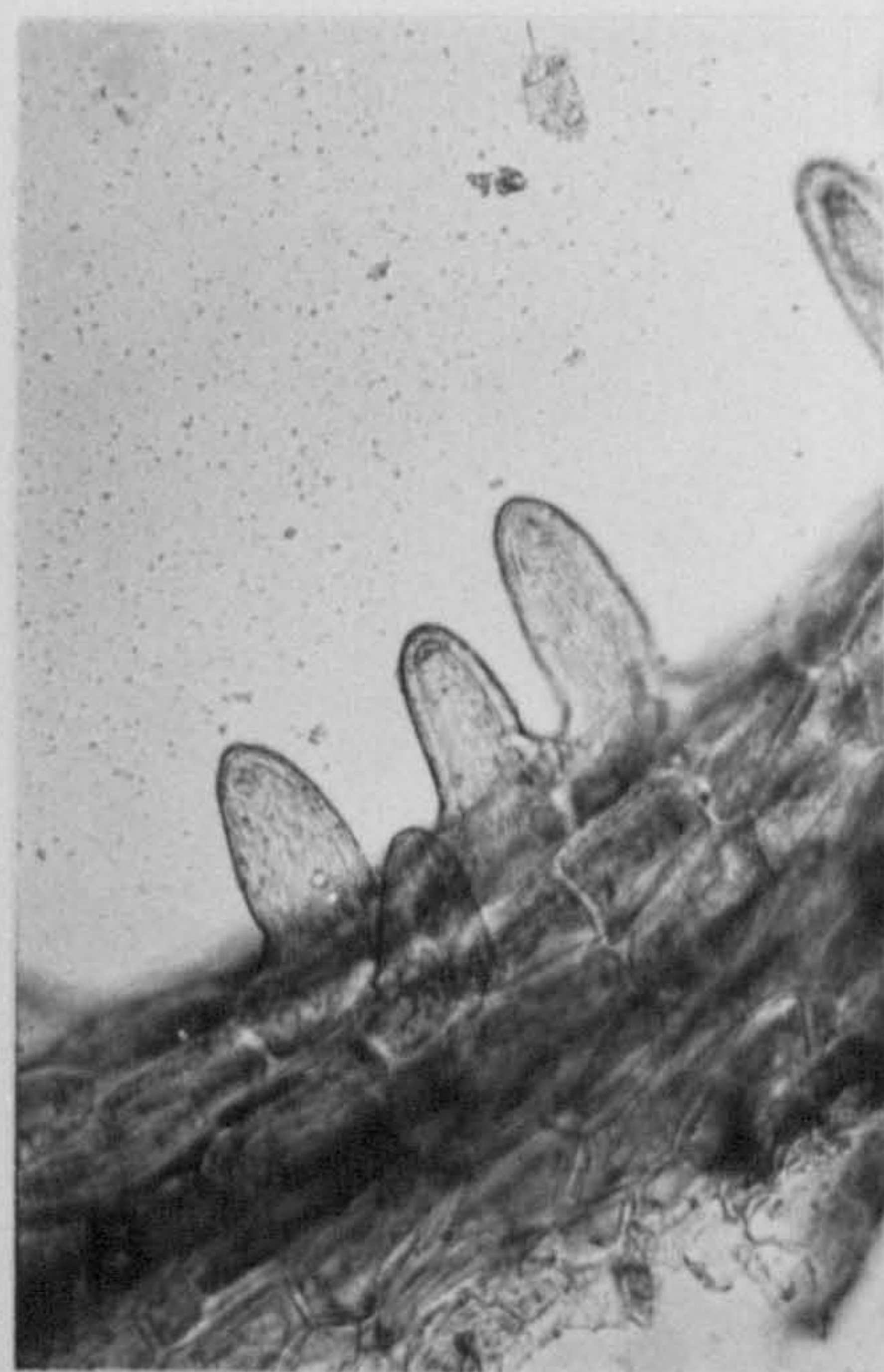
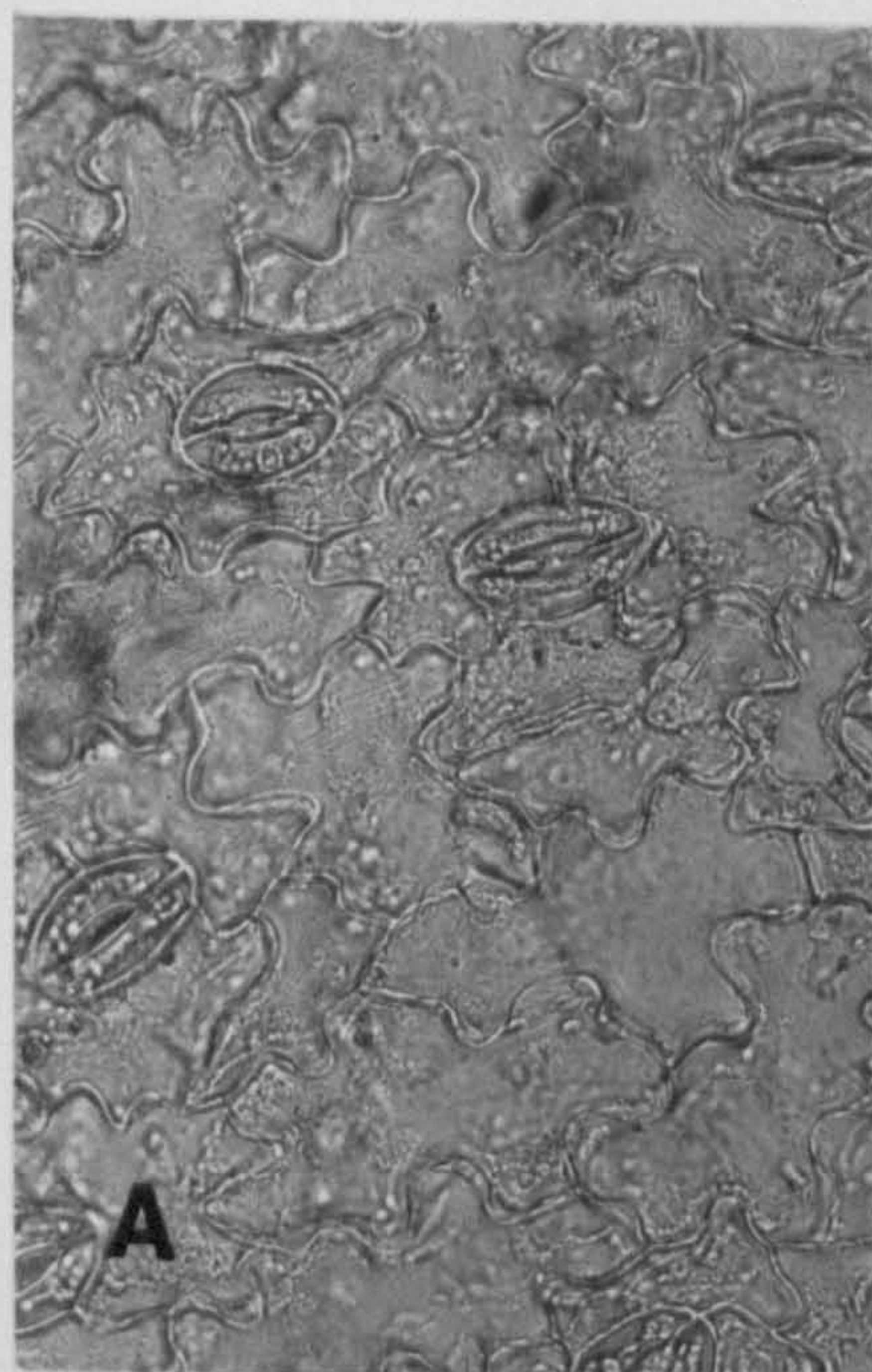


Plate 5. 15 Artificial *R. japonica* var. *compacta* x *F. baldschuanica* P102b

Note the unusual hairs and their arrangement in **B**

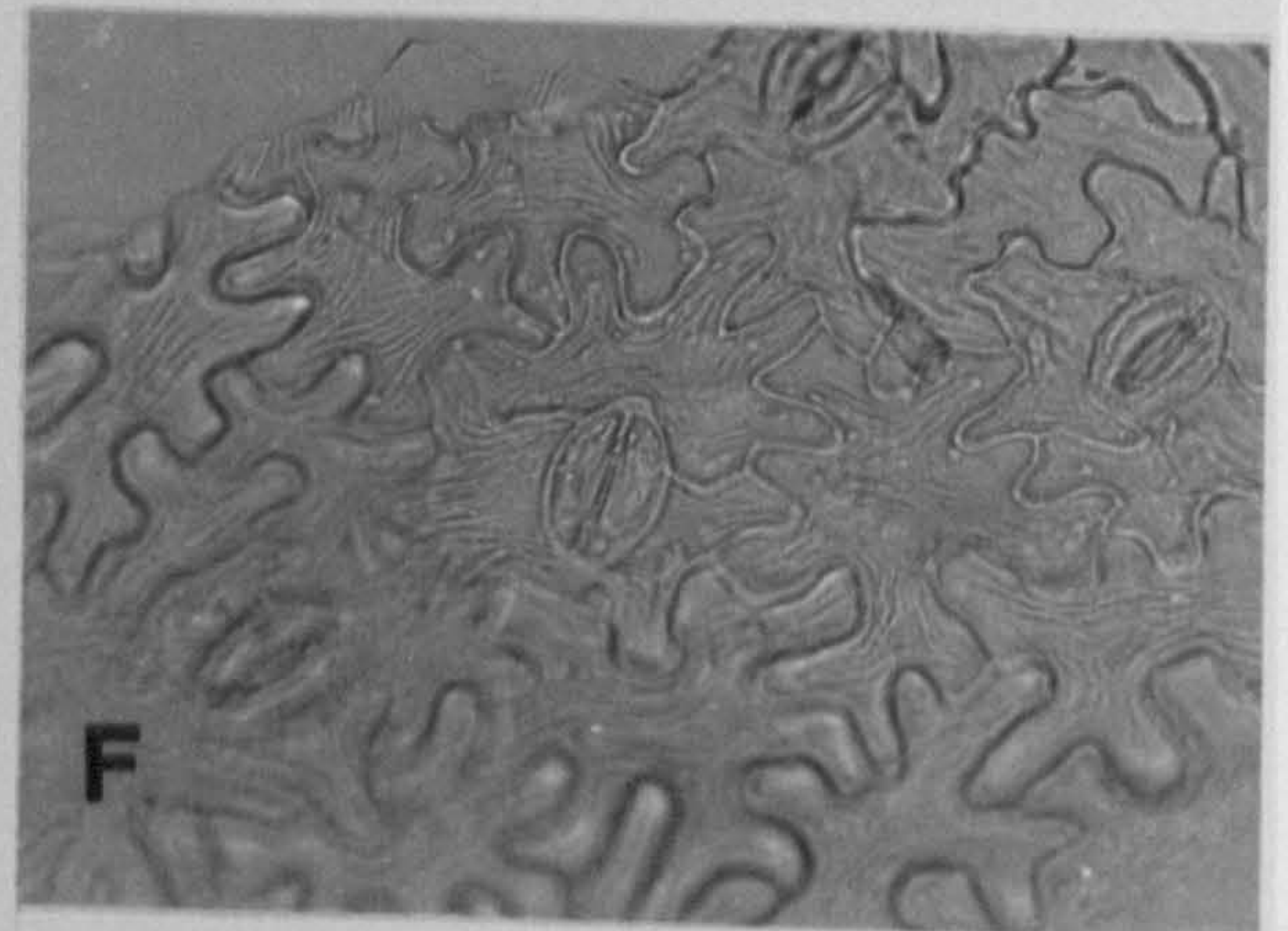
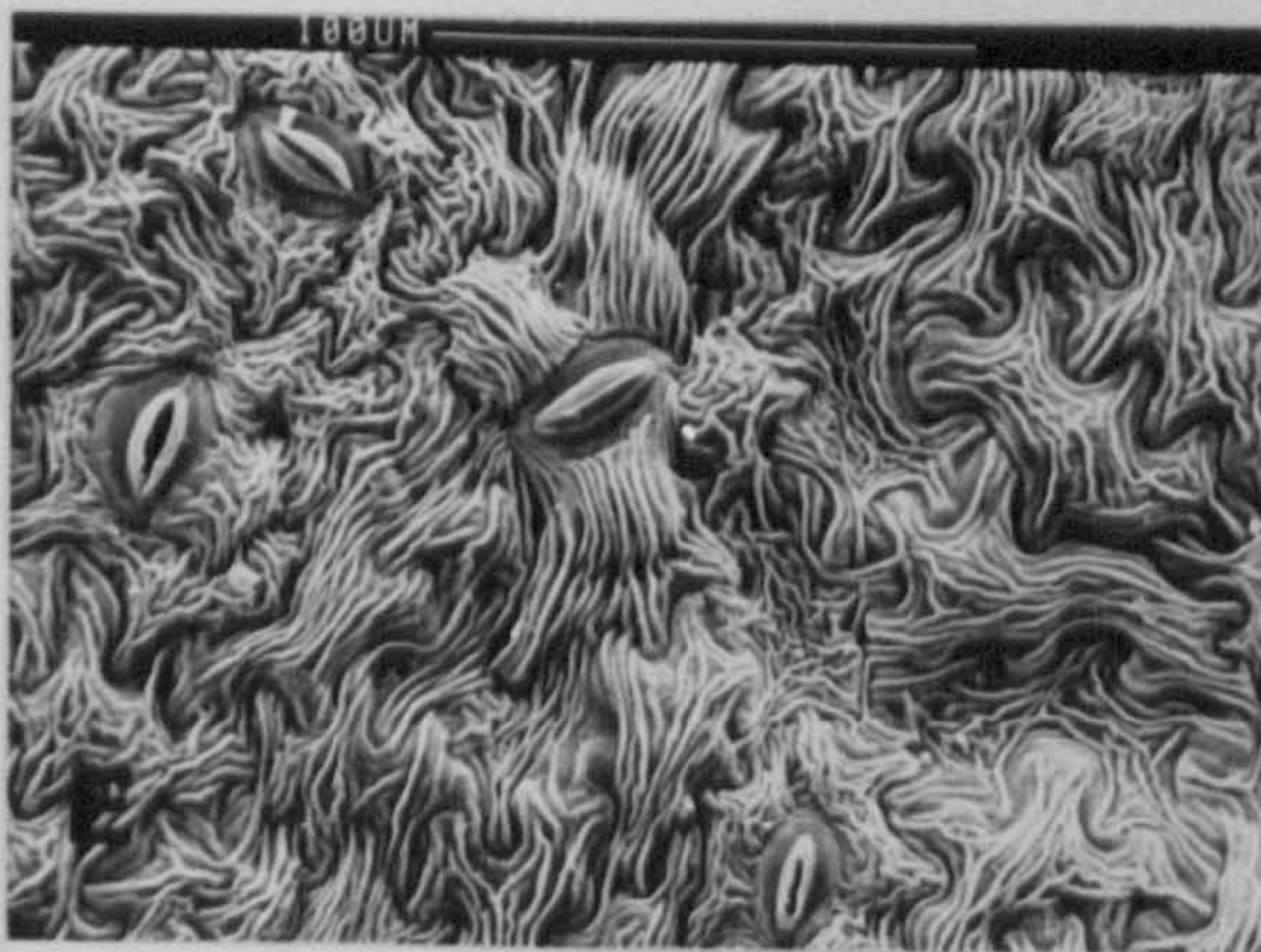
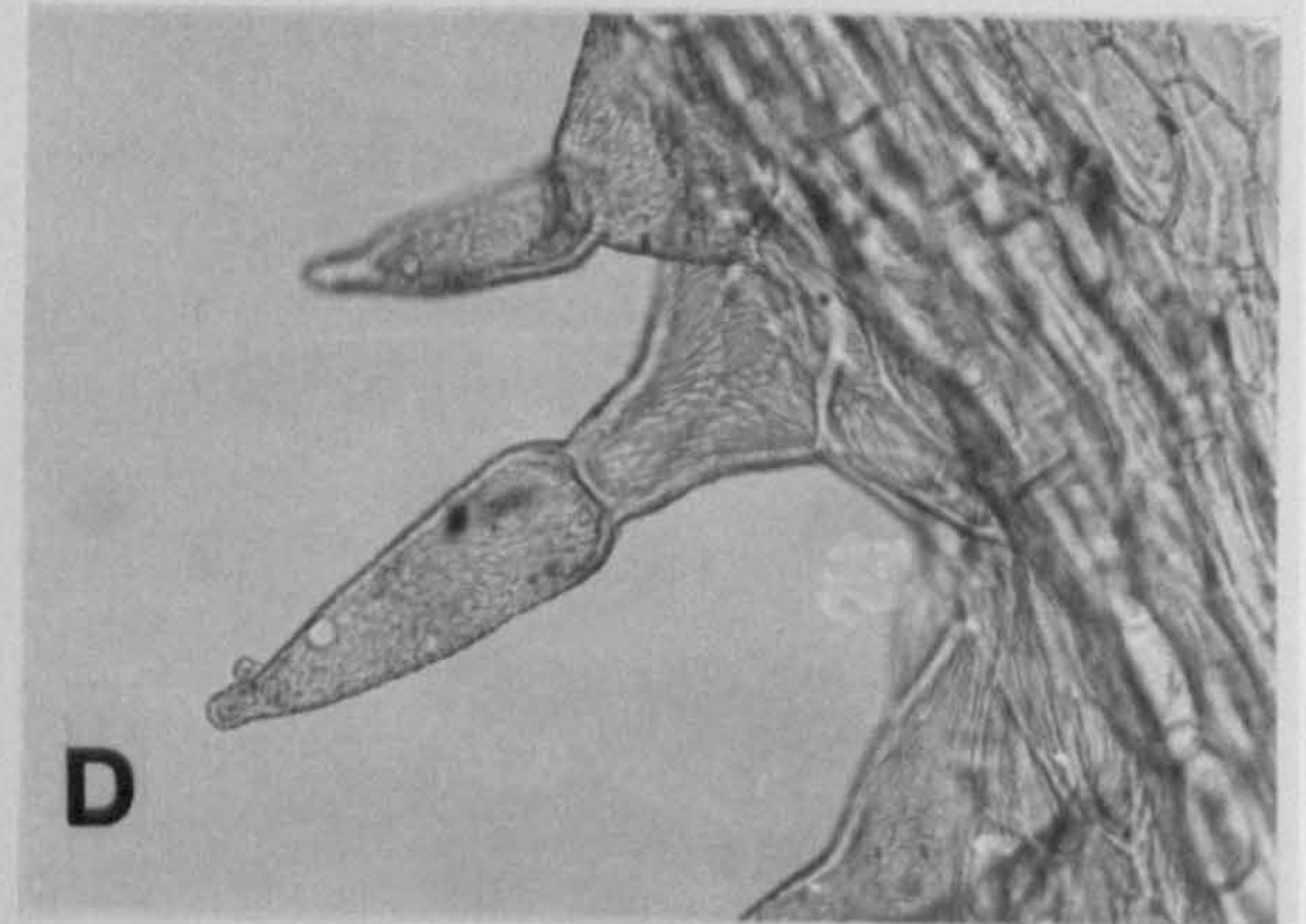
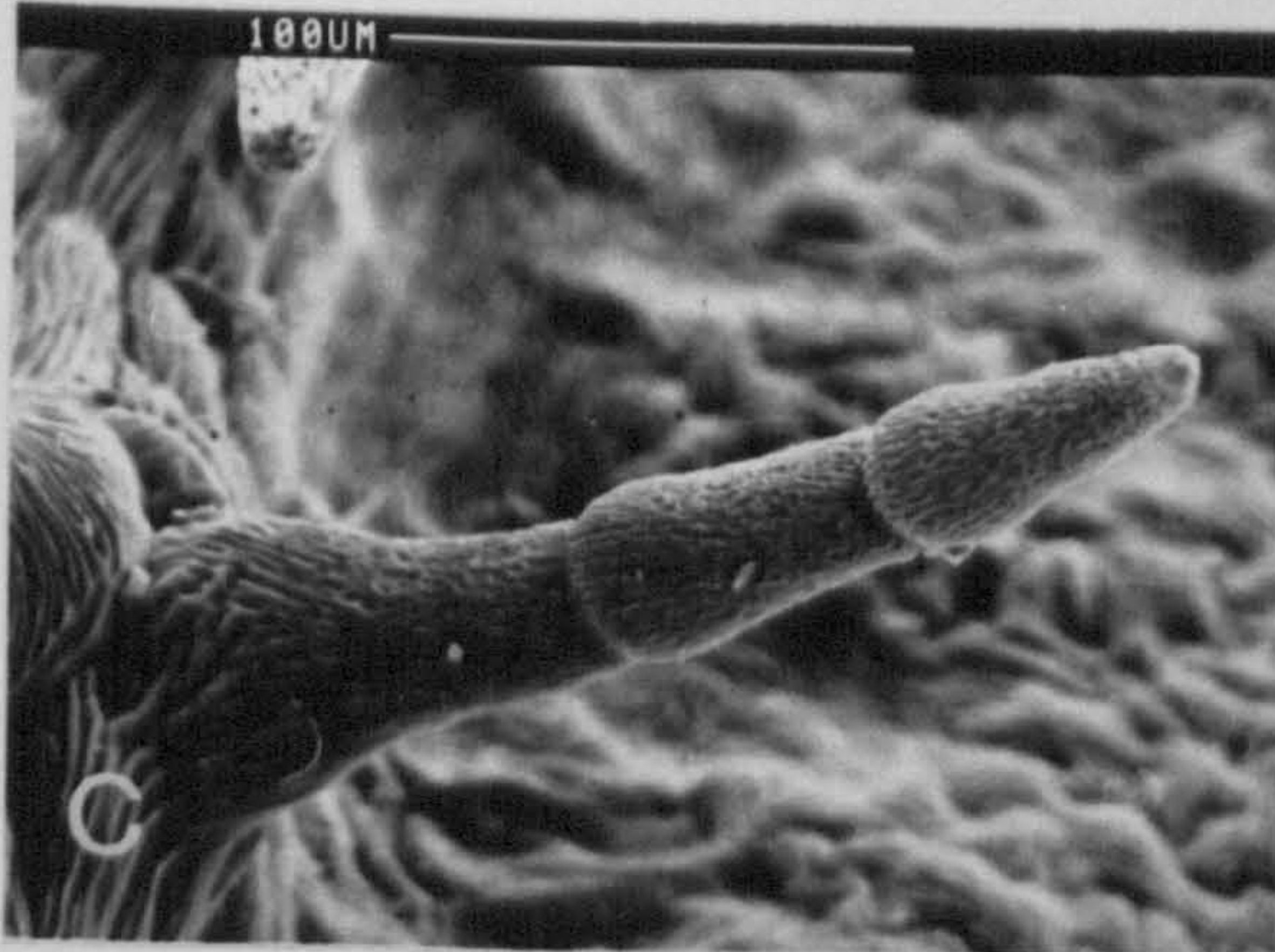
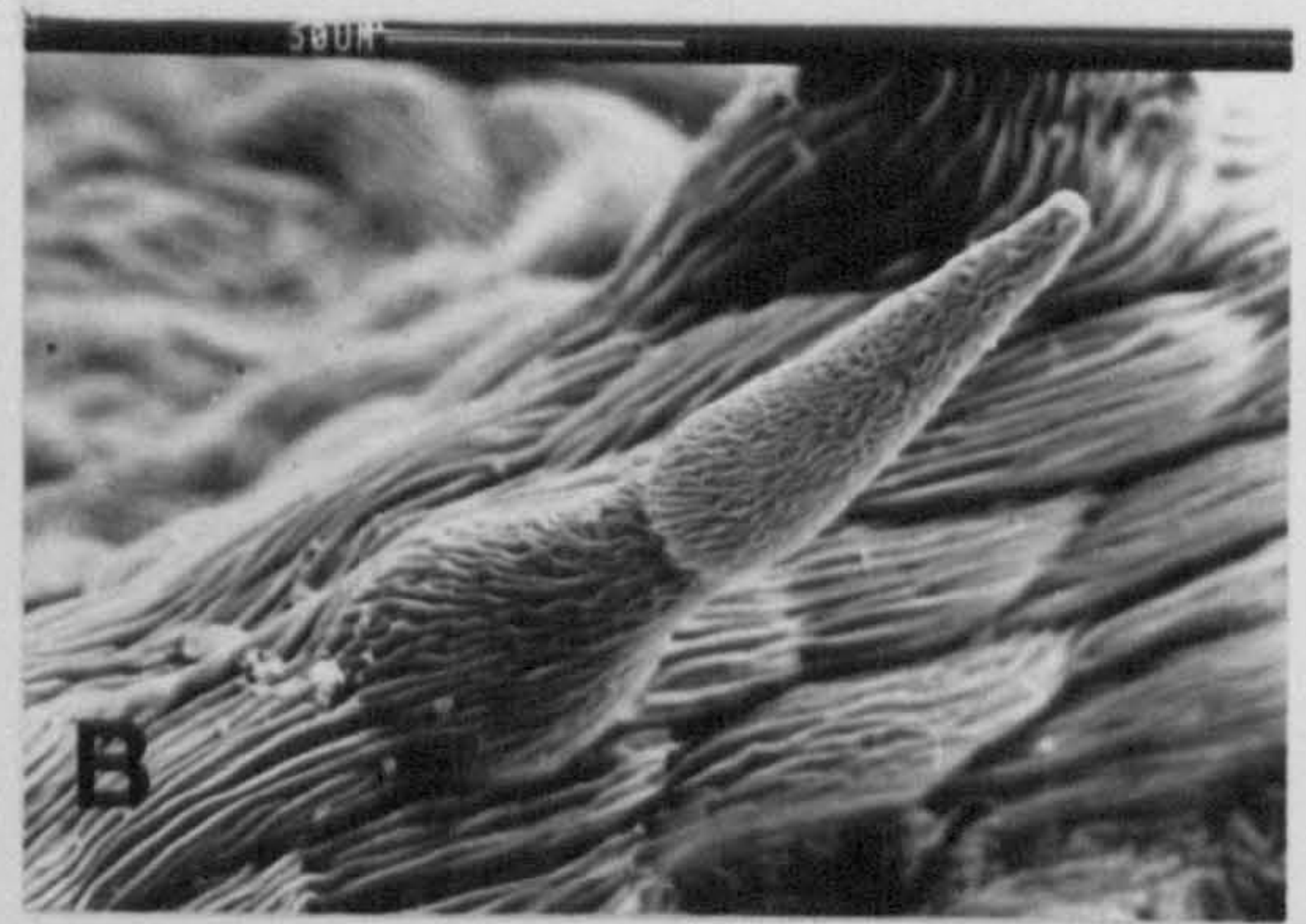
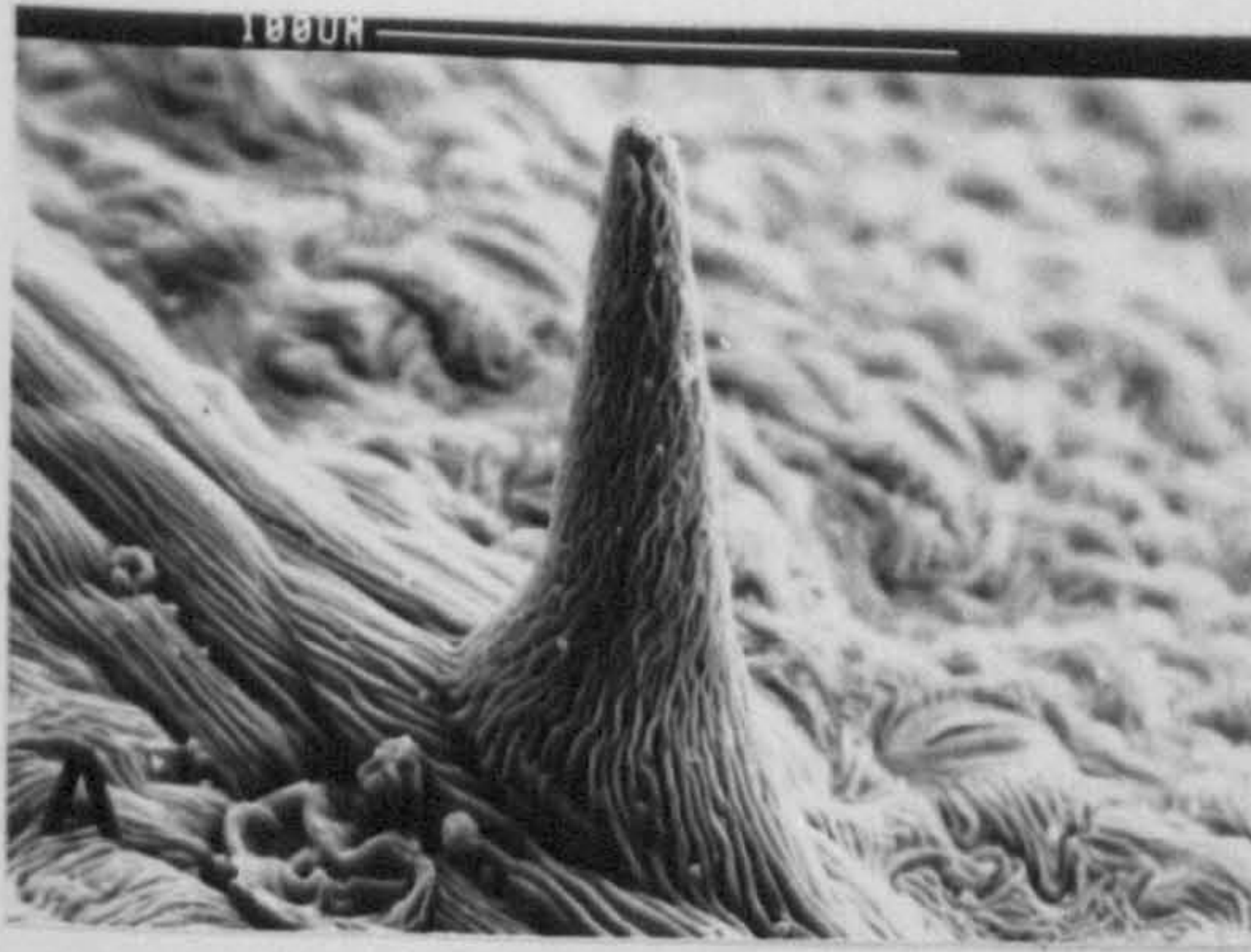


Plate 5.16 Tetraploid interspecific artificial *Reynoutria* hybrids

A,B,C,E *R.sachalinensis* x *R. japonica* var. compacta P79c, note particularly the curiously shaped hair in C

D,F *R.japonica* var. compacta x *R. sachalinensis* P78a

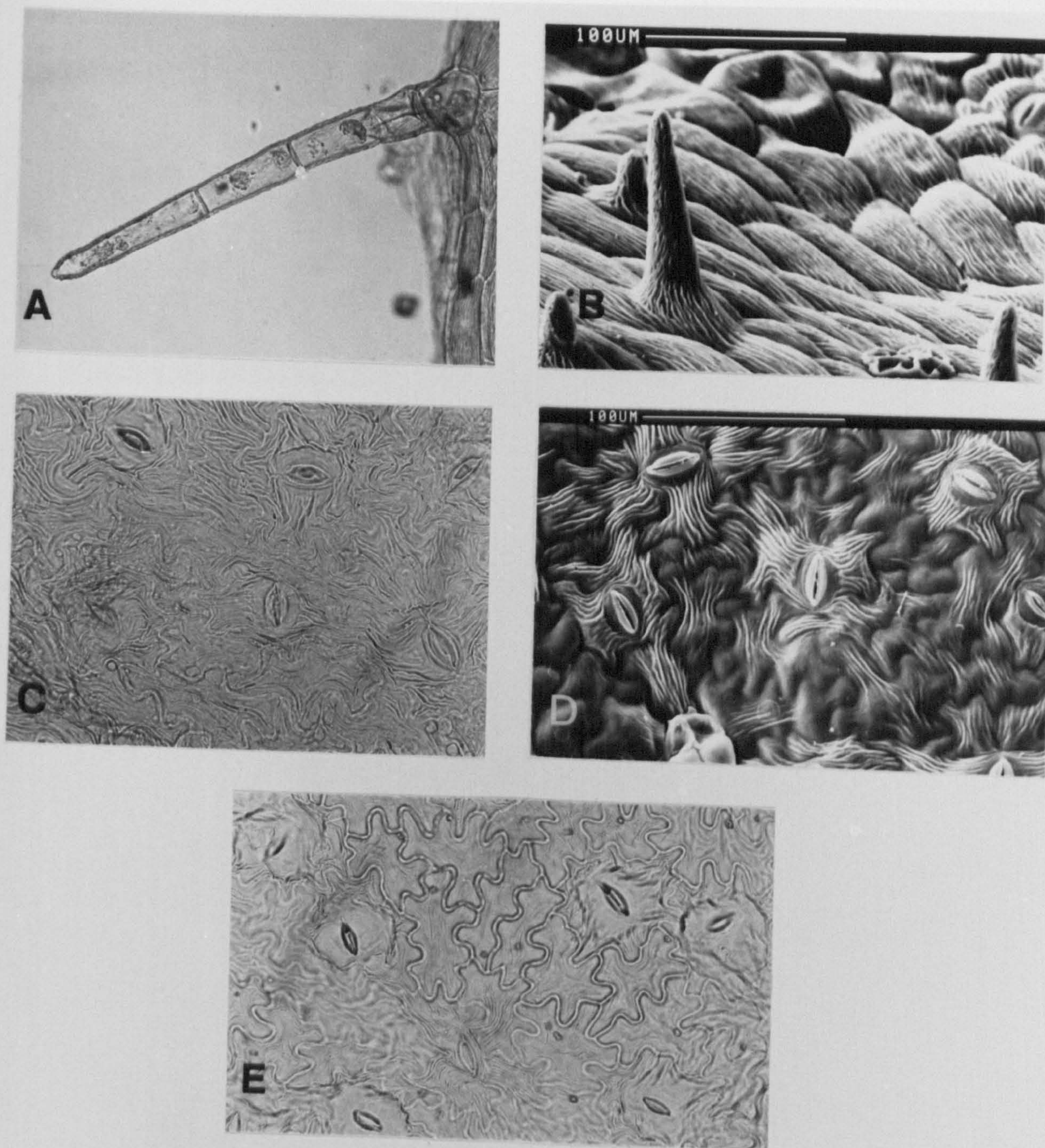


Plate 5.17 Tetraploid interspecific wild *Reynoutria* hybrids

A - D *R. japonica* var. *compacta* X *R. sachalinensis* P13 -- note variation in degree of striation between **C** and **D**

E *R. japonica* var. *compacta* X *R. sachalinensis* P125

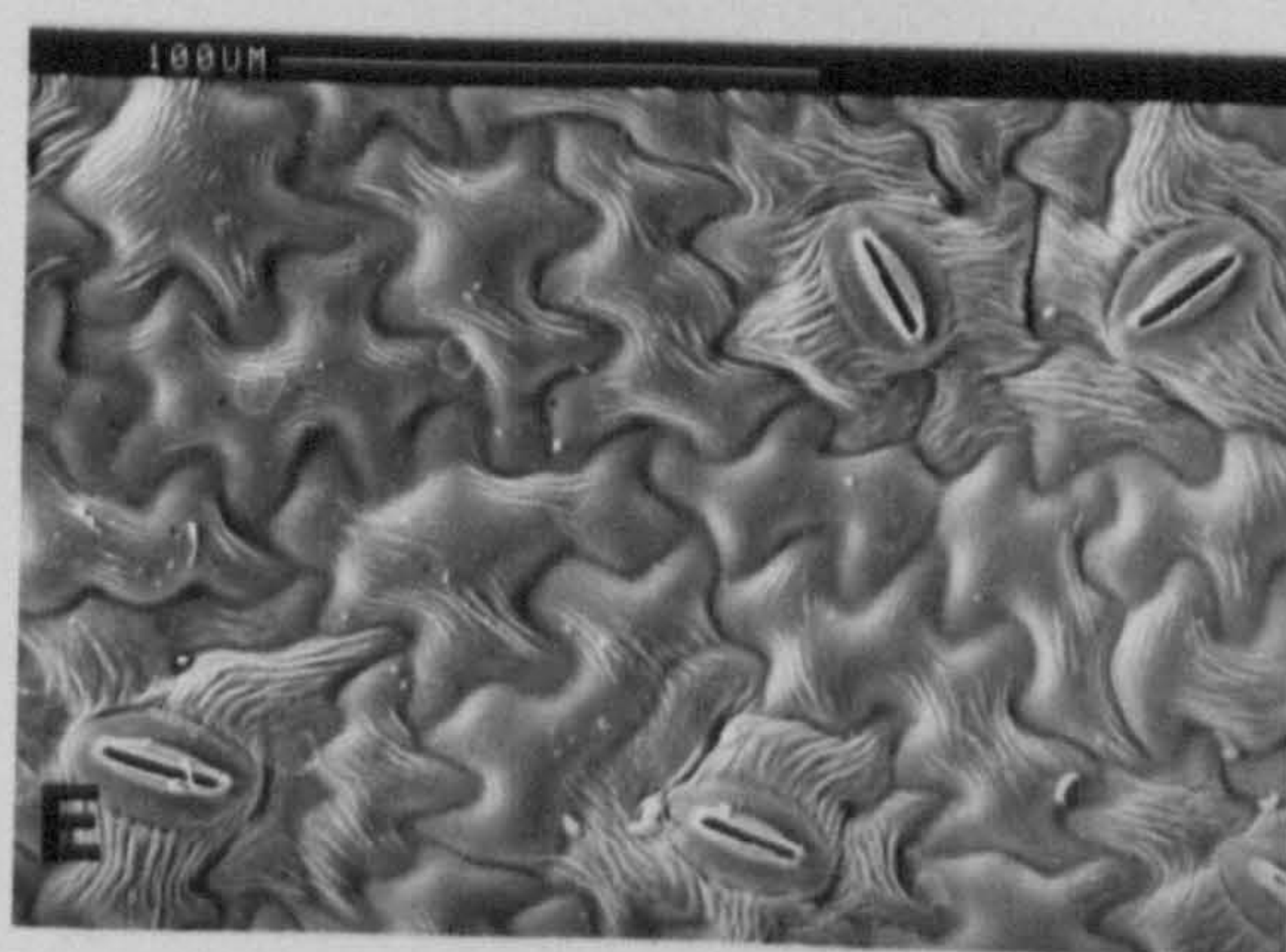
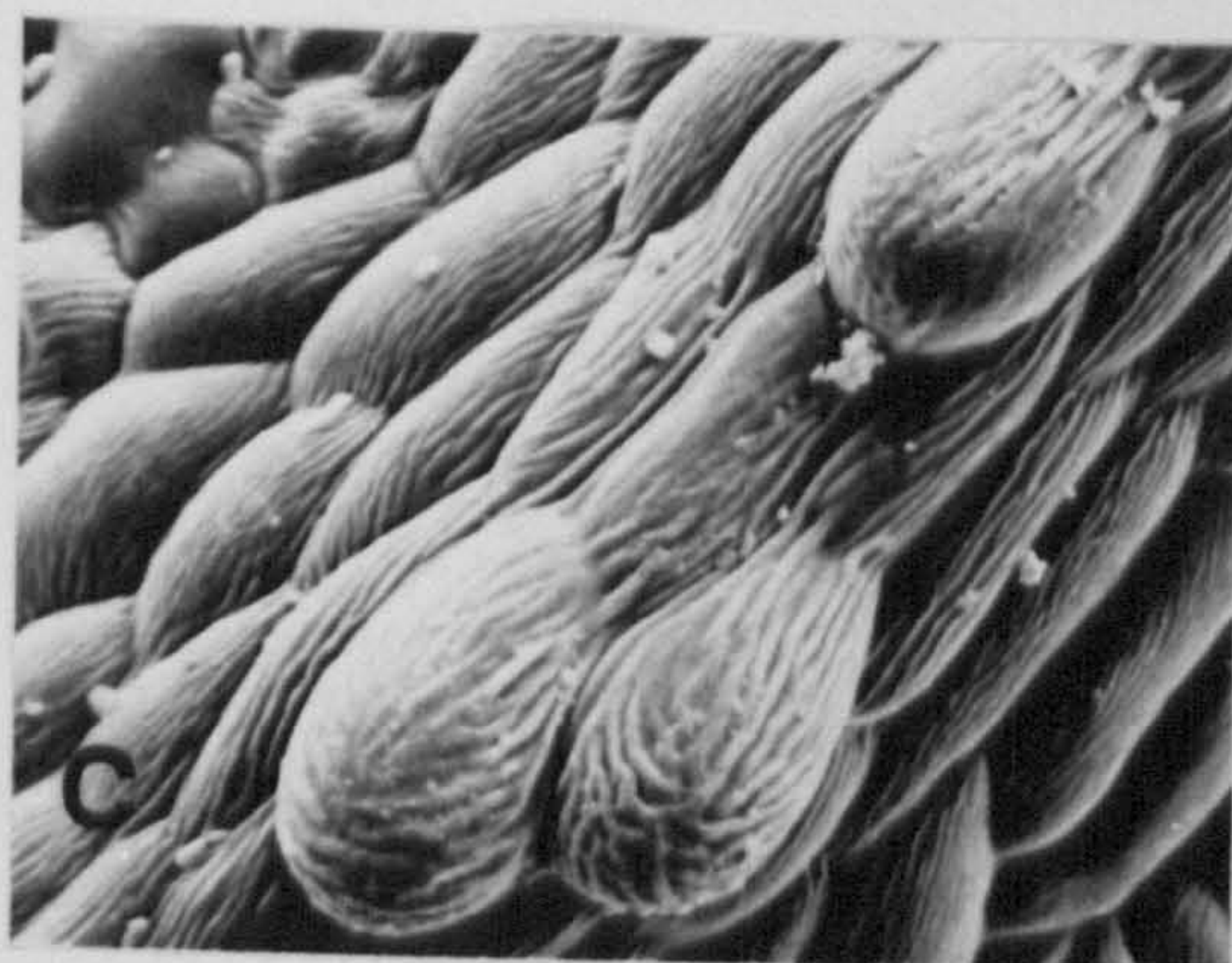
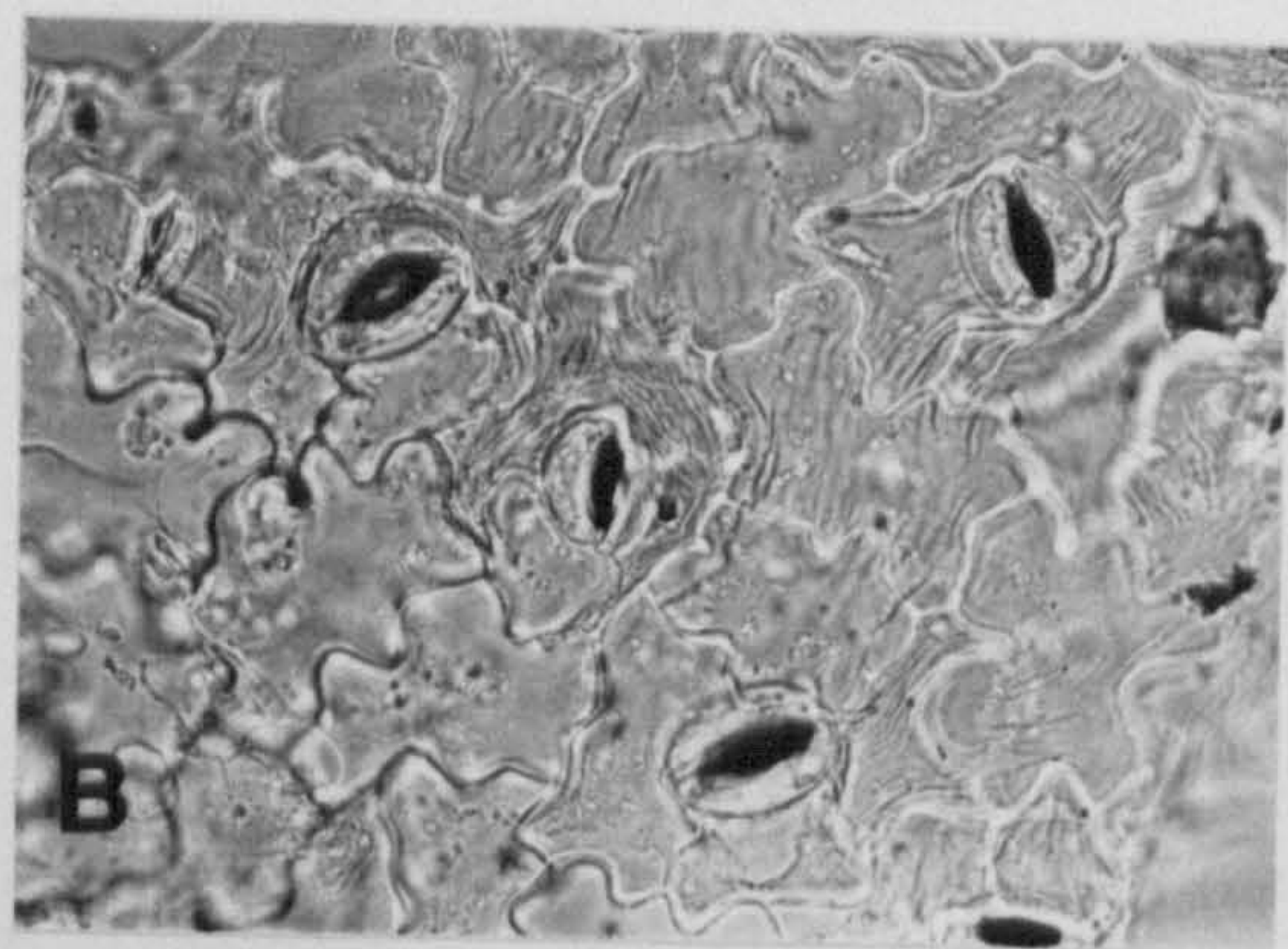
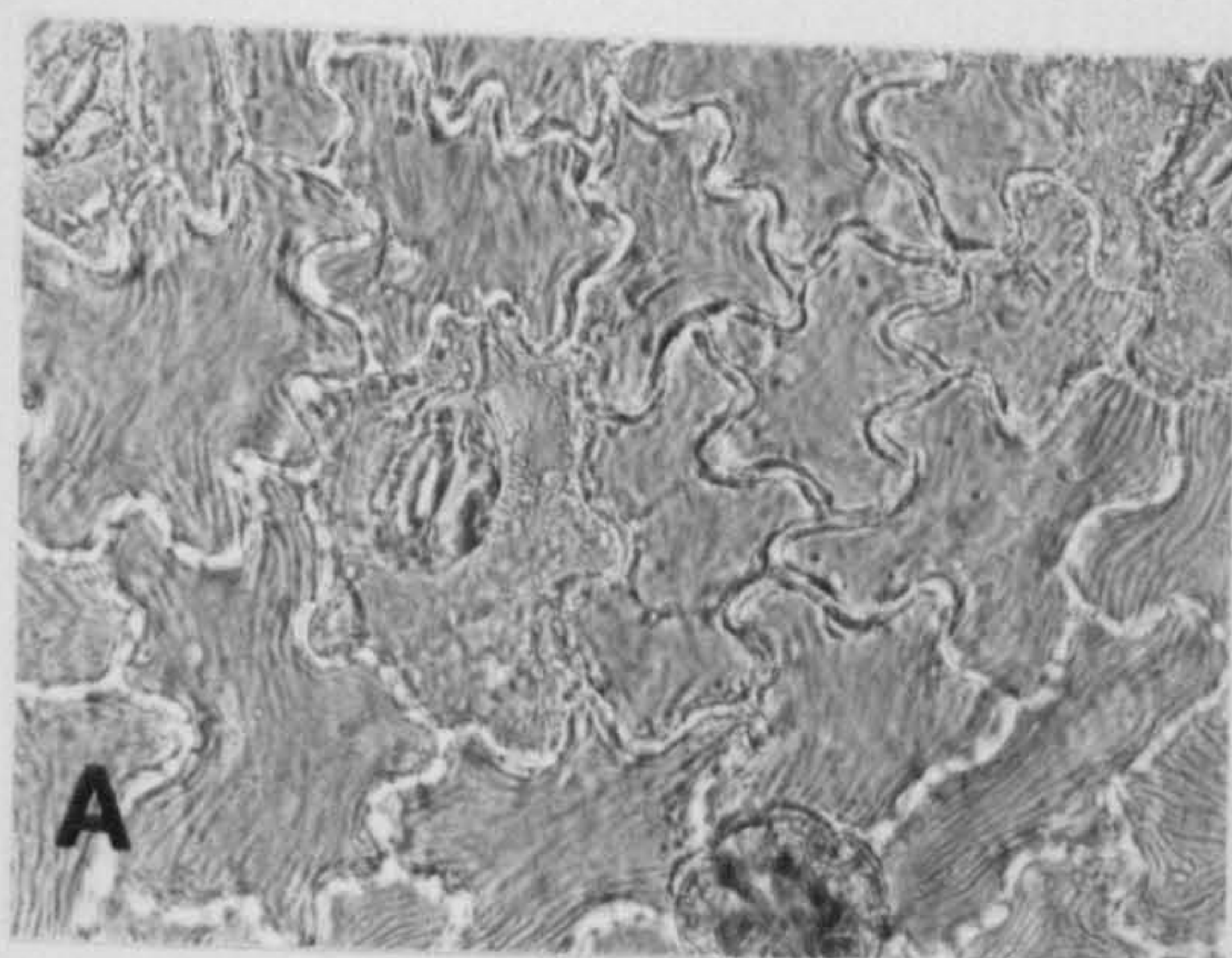


Plate 5. 18 *R. japonica* var. *japonica* x *F. baldschuanica*
hybrids

A Artificial hybrid P94b

B P83b

C P80a

D,E P80d

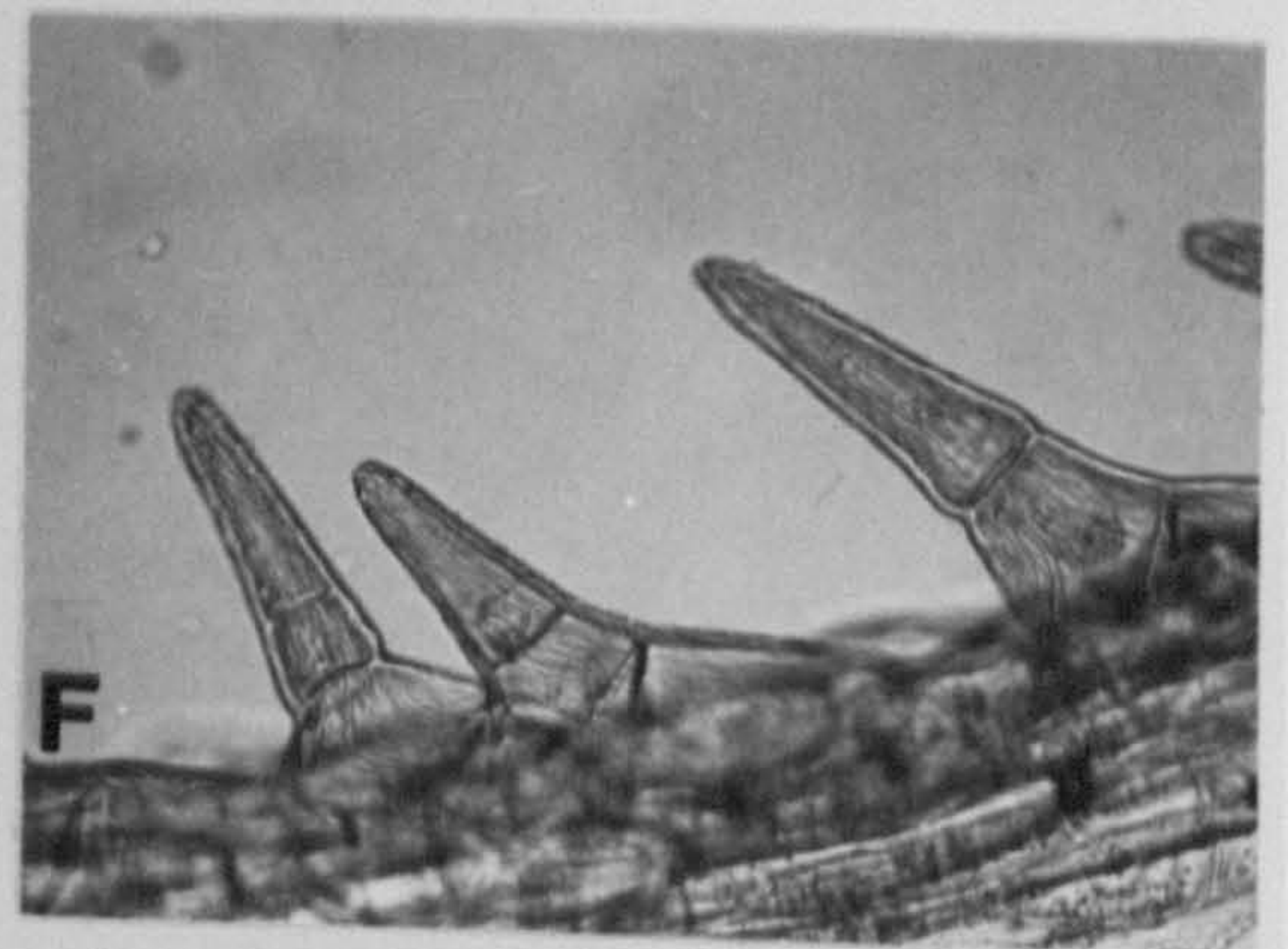
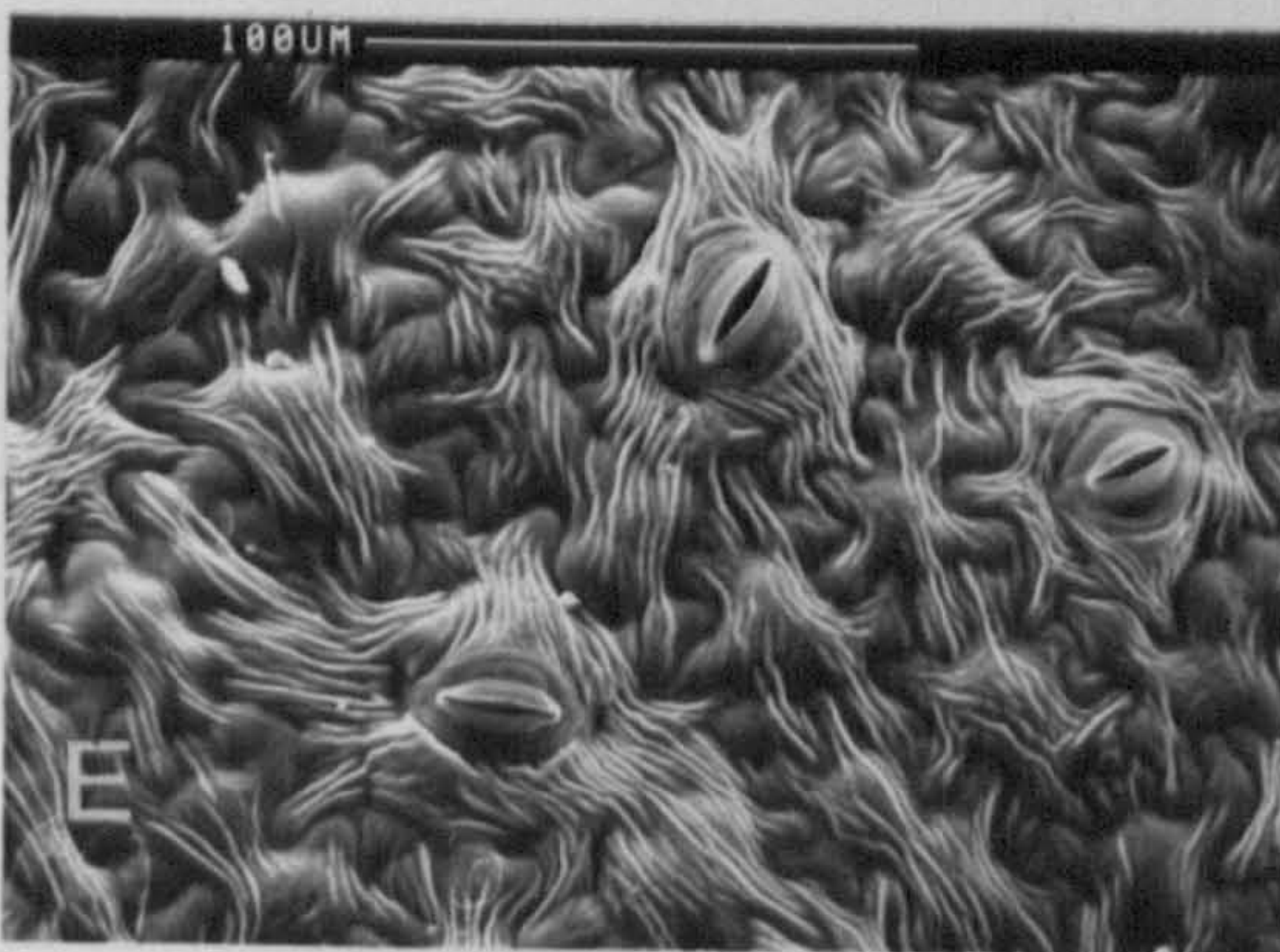
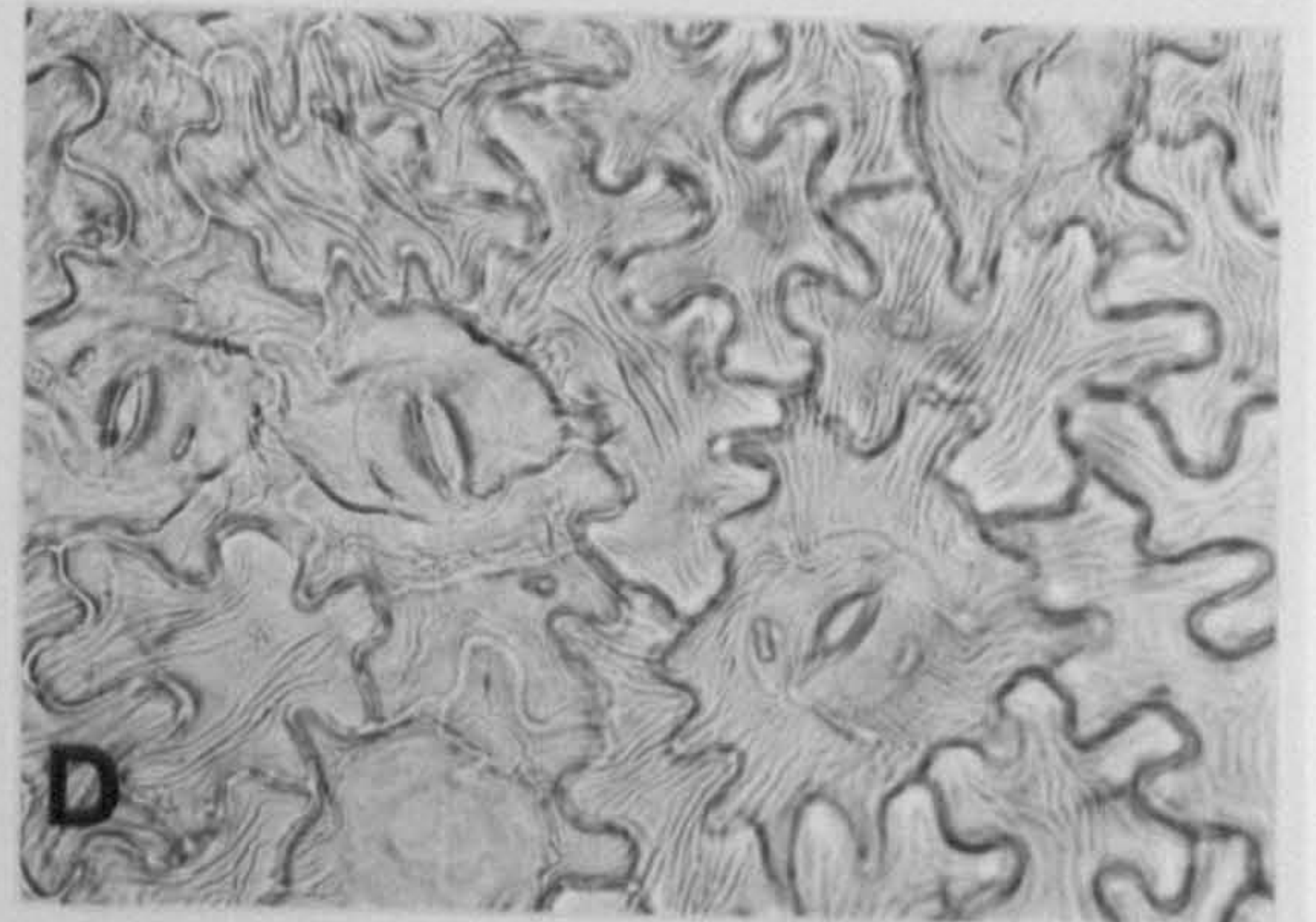
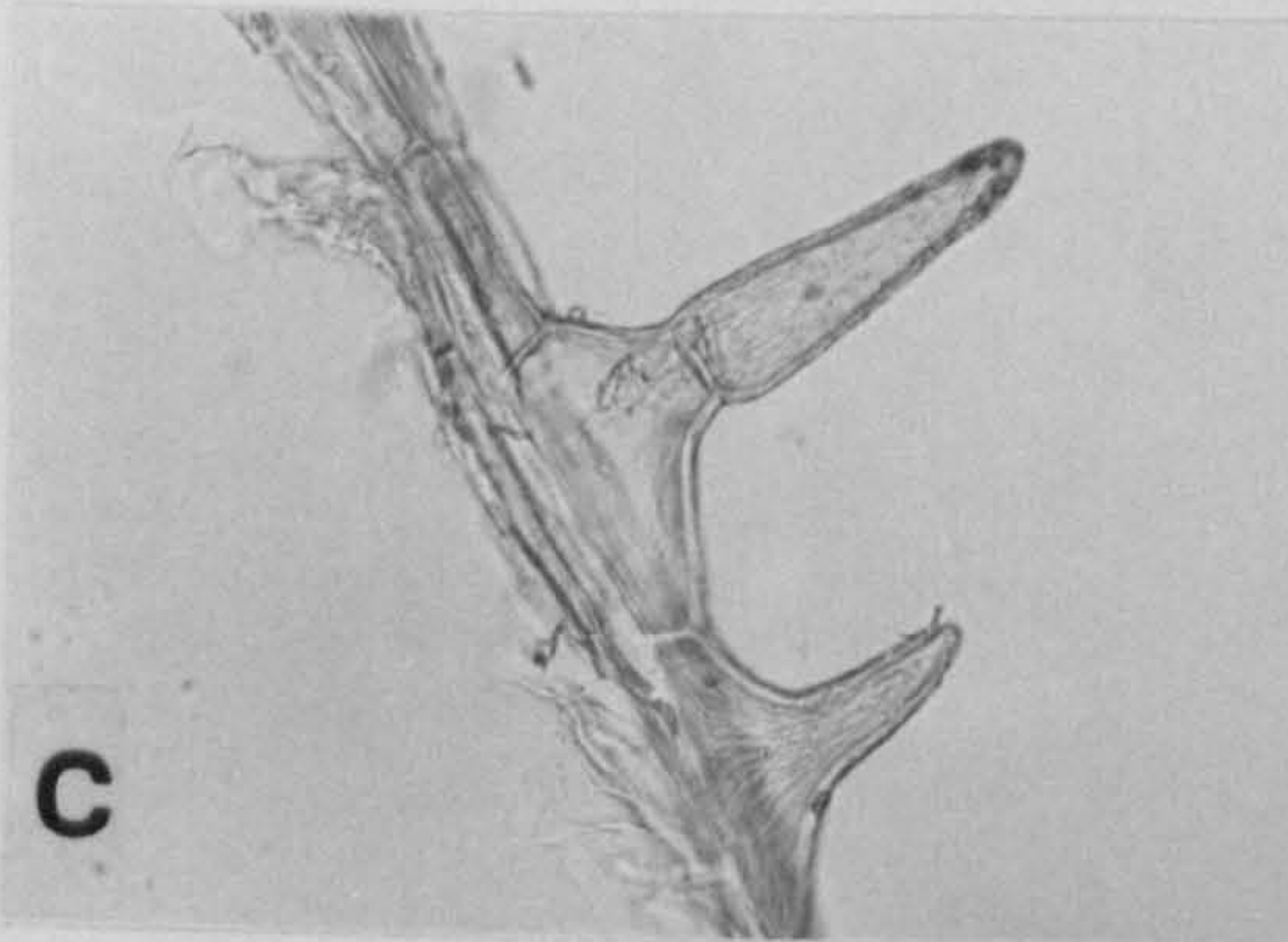
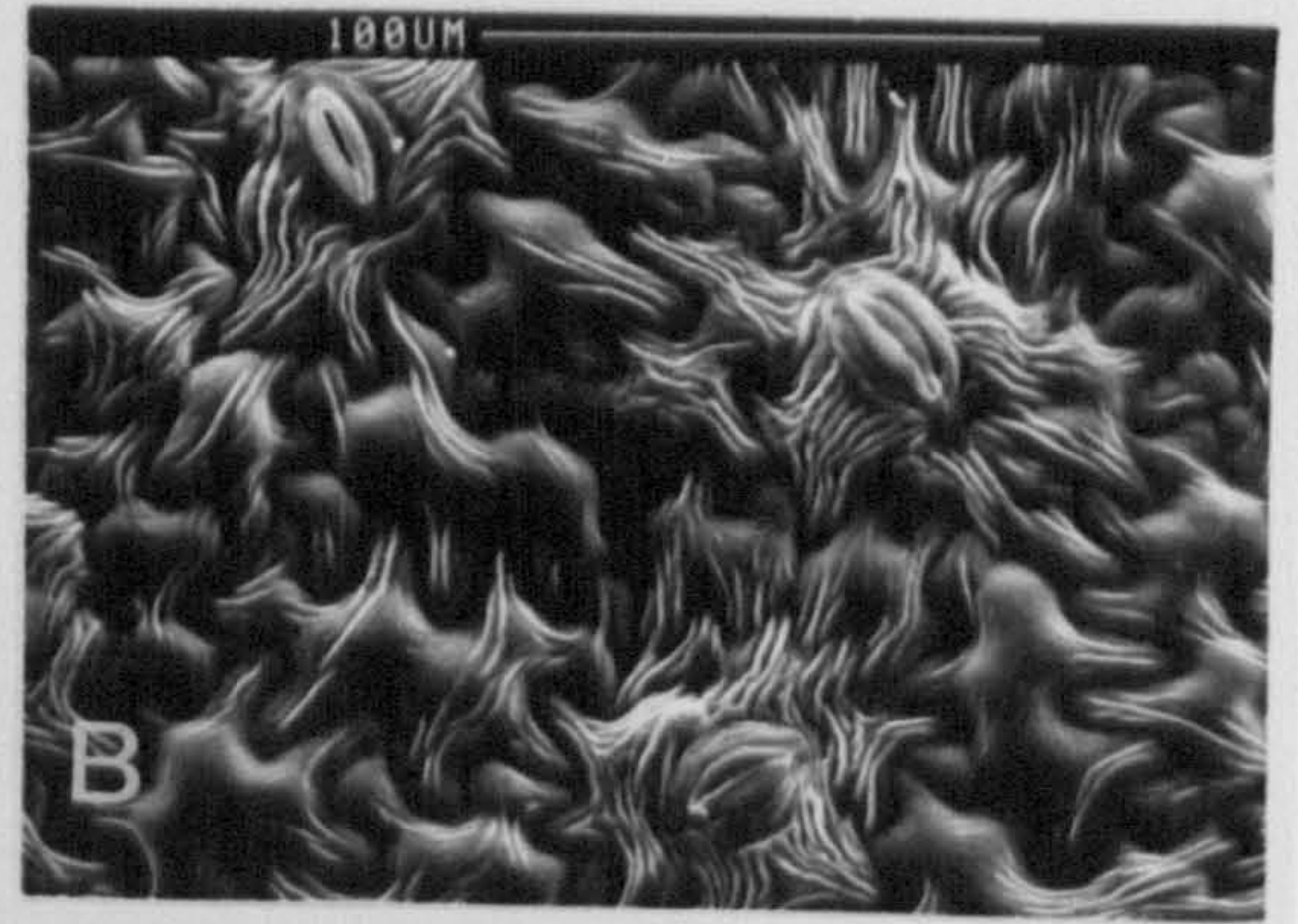
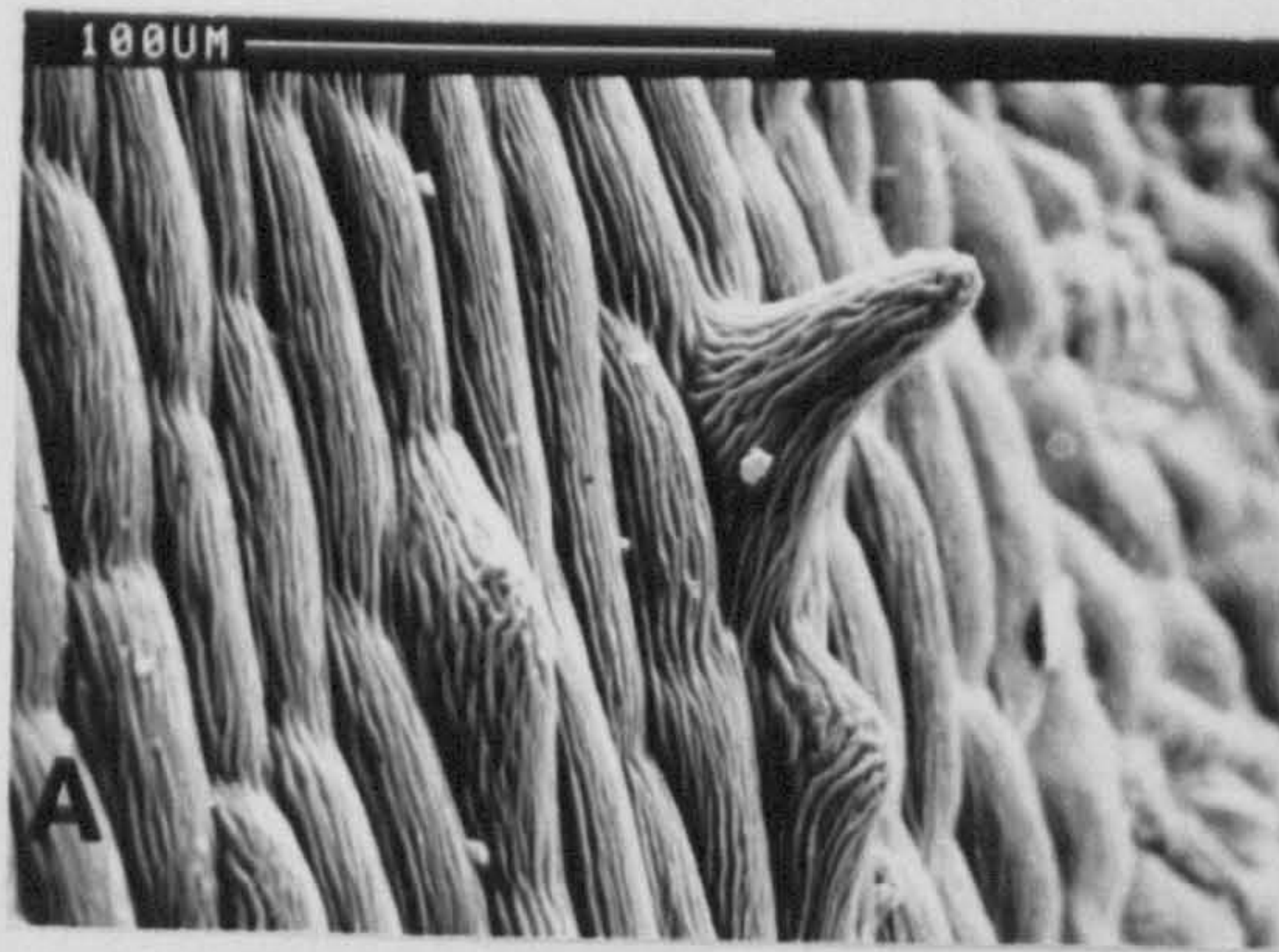


Plate 5. 19 Hexaploid *R. japonica* x *R. sachalinensis* hybrids

A - C Artificial hybrid P75b

D Artificial hybrid P75c

E Putative hybrid P32

F Putative hybrid P31

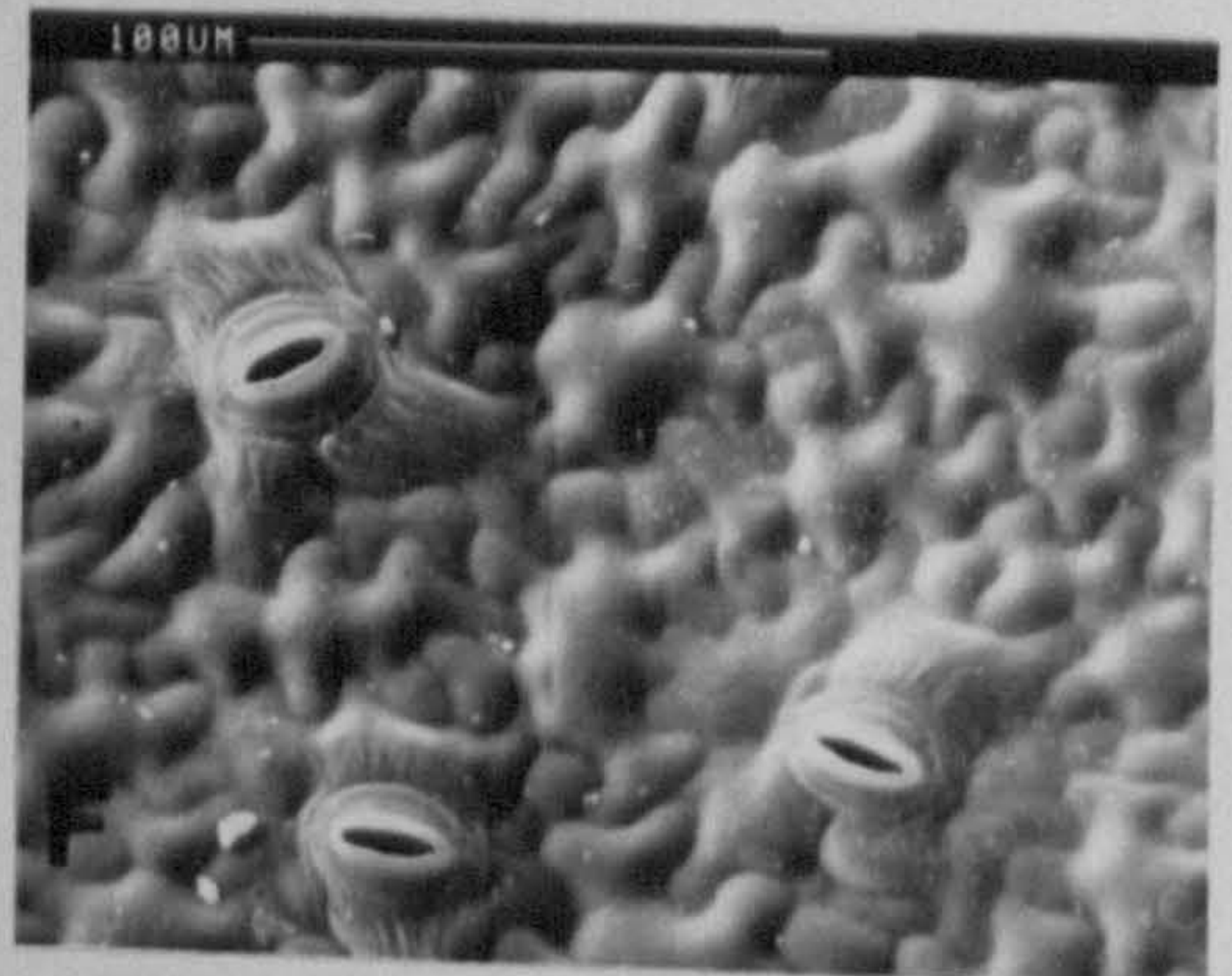
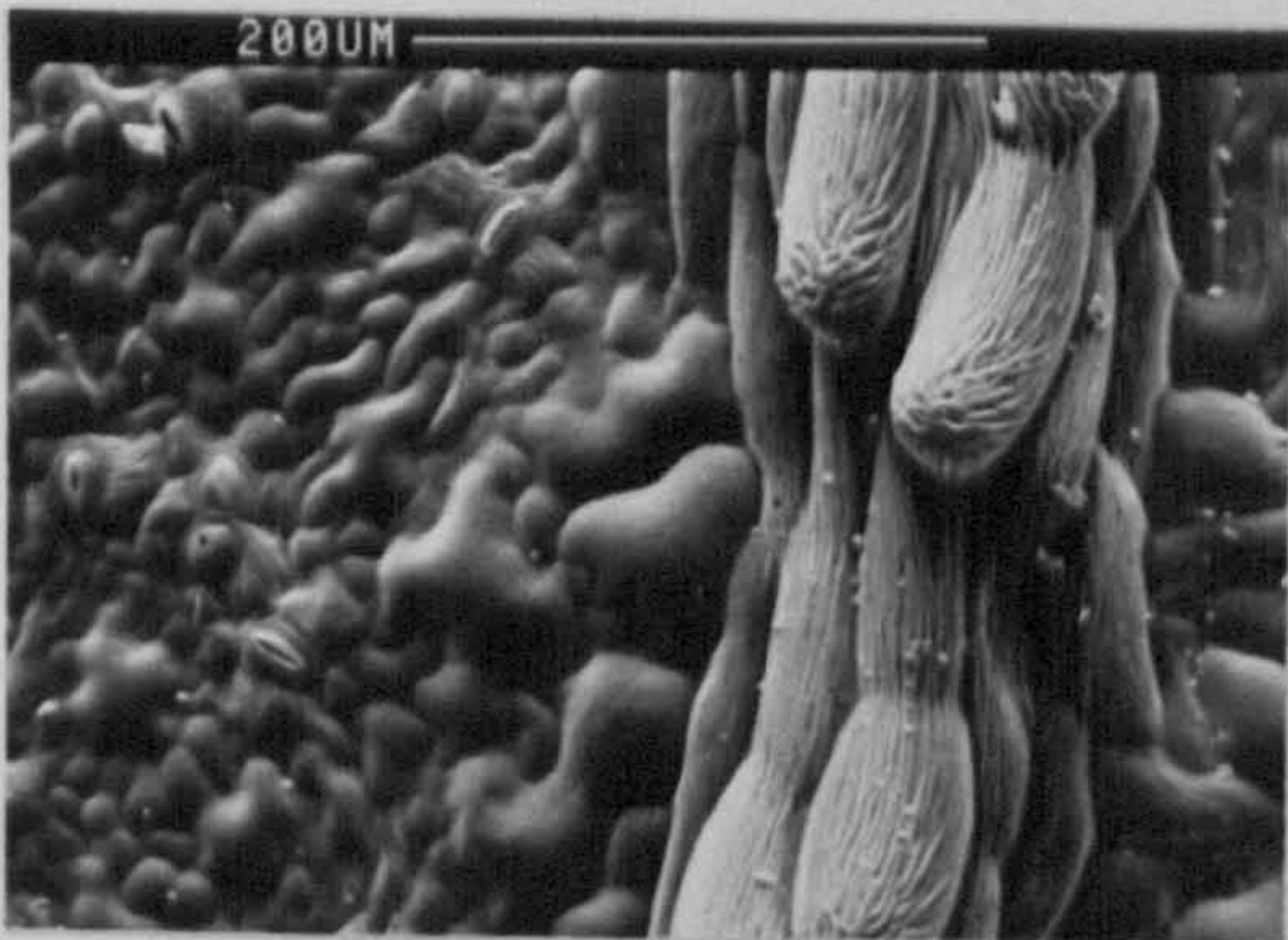
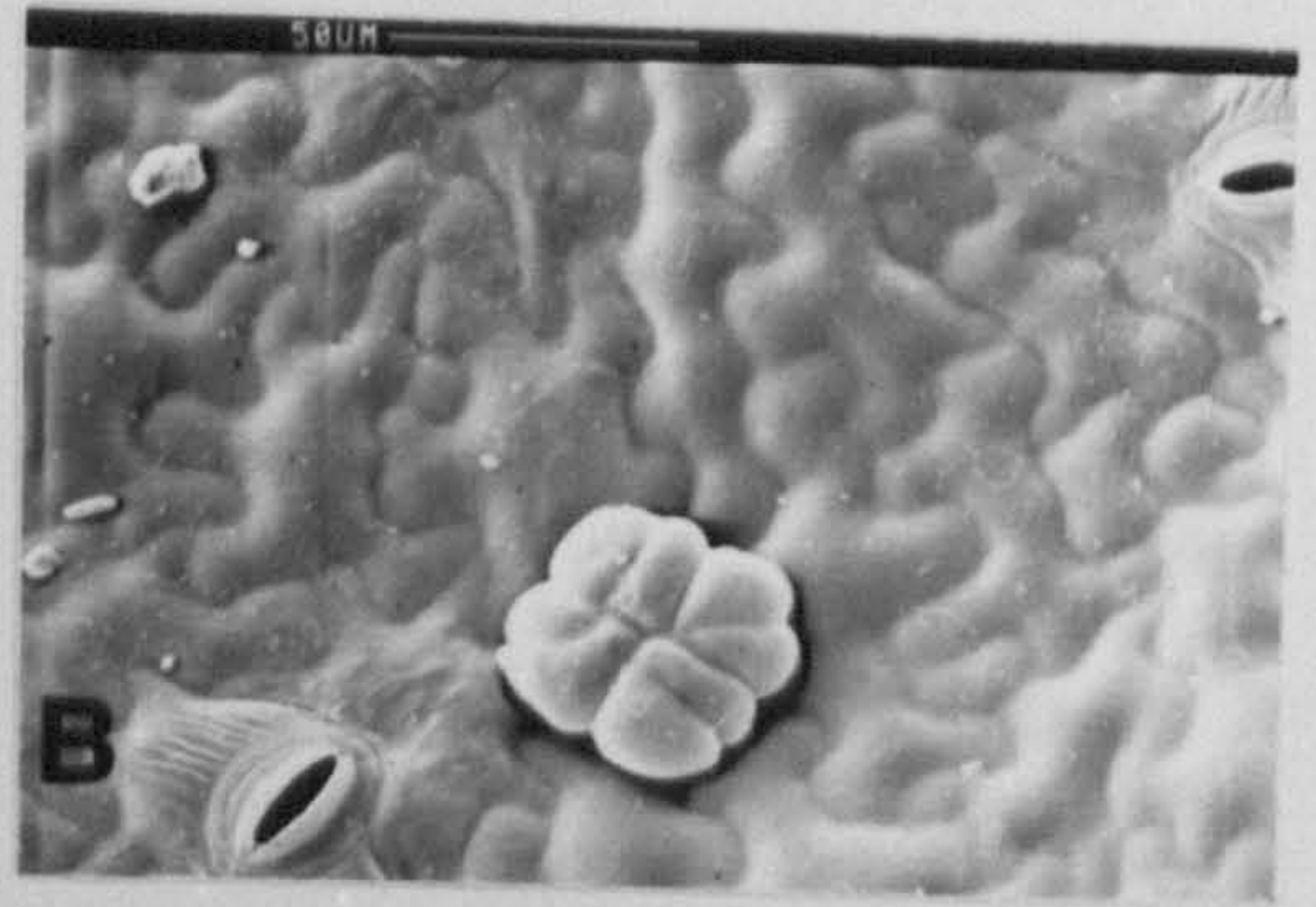
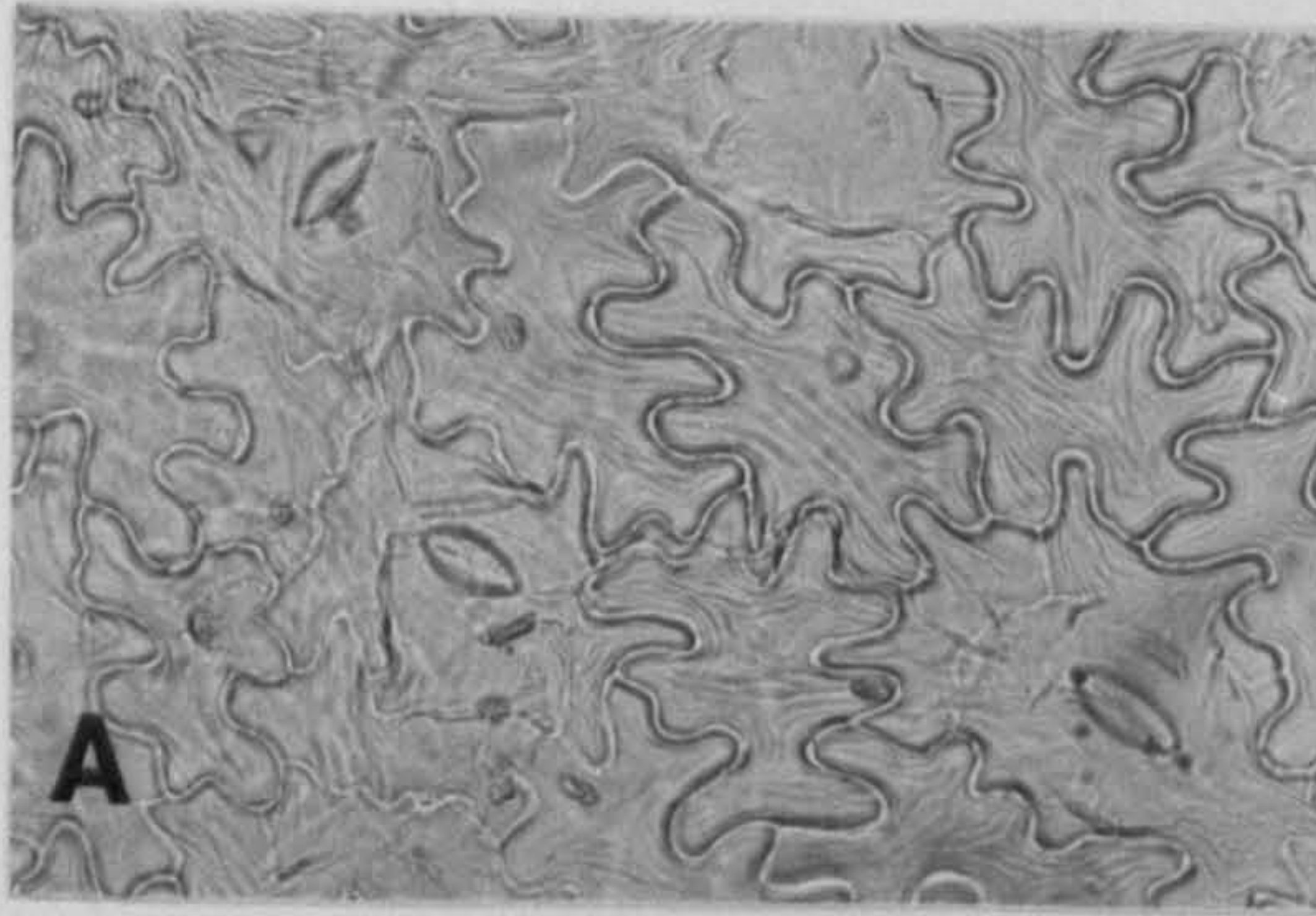


Plate 5. 20 Hexaploid *Reynoutria* hybrids

A Putative *R. japonica* x *R. sachalinensis* P16

B - F Artificial *R. japonica* var. *japonica* x *R. japonica* var. *compacta* P76a,b

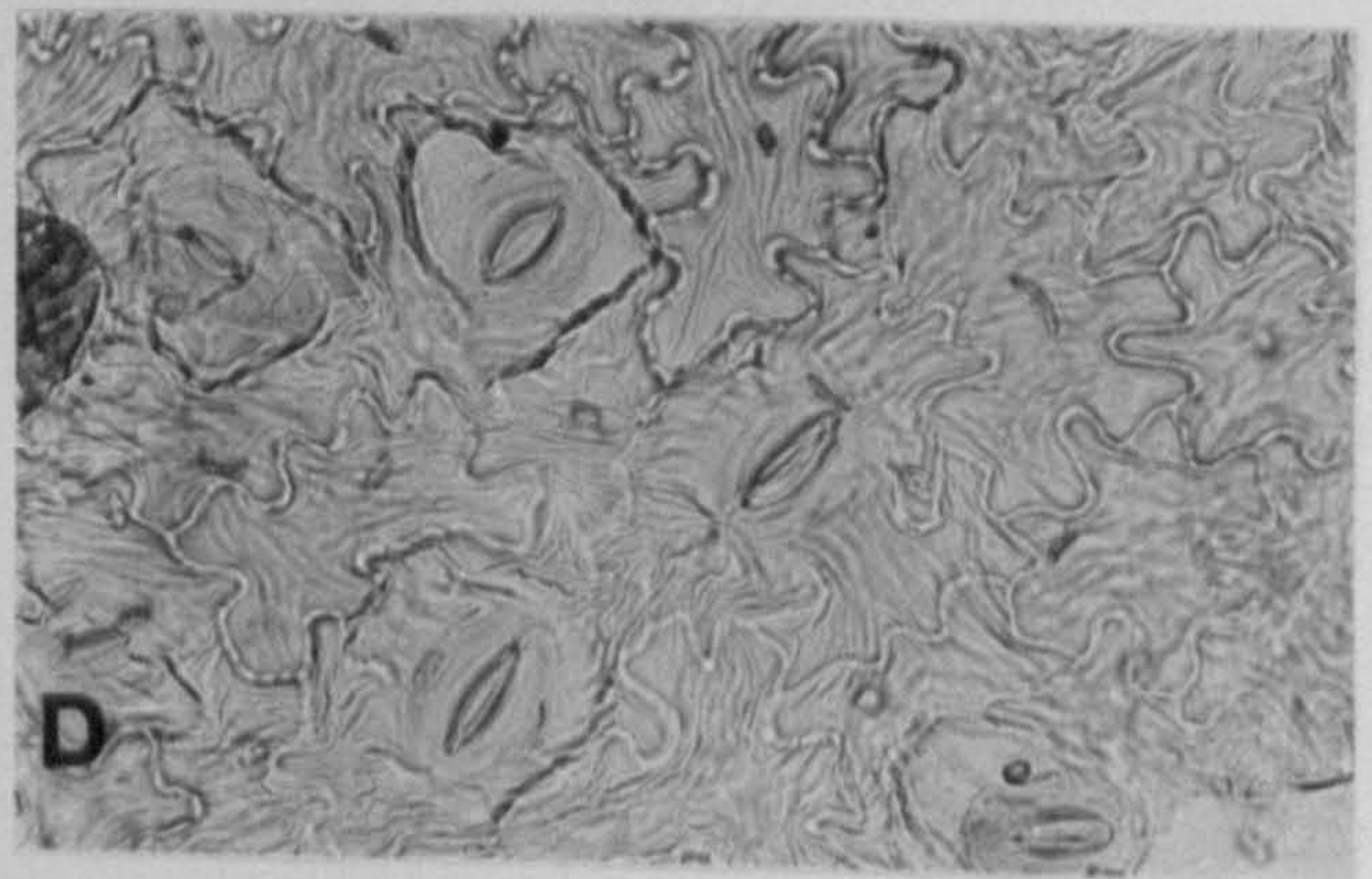
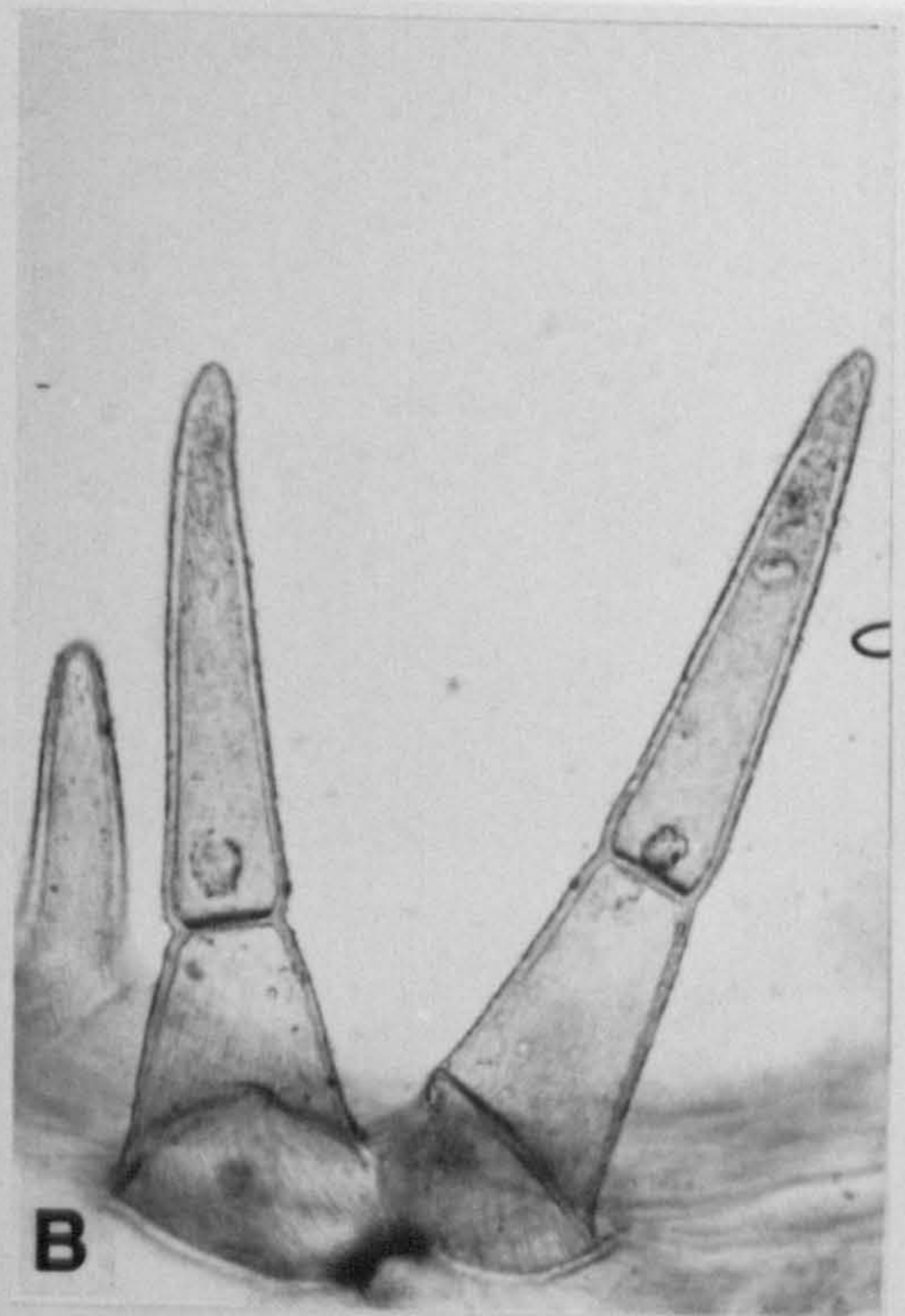
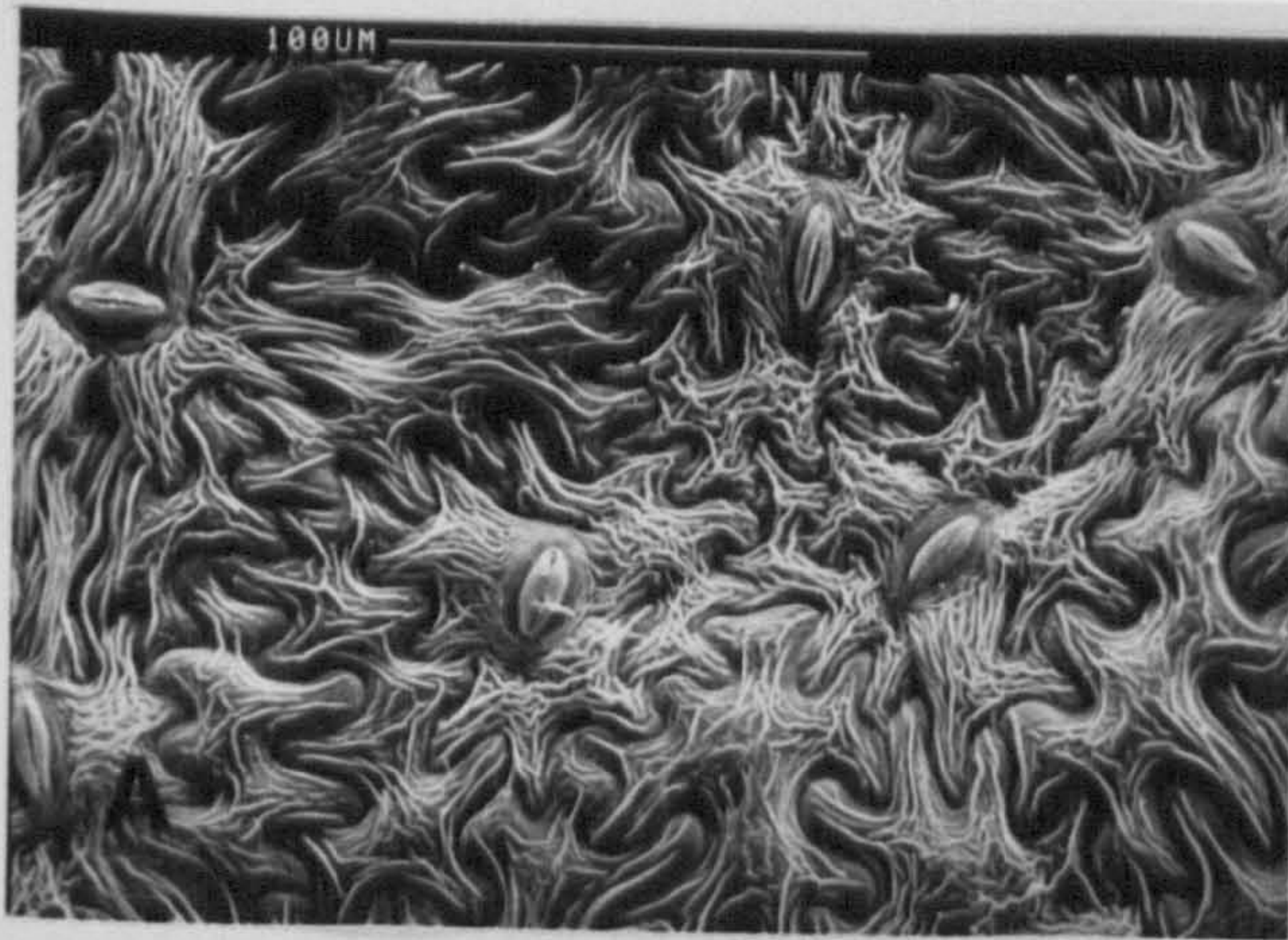


Plate 5. 21 Octoploid putative *R. japonica* x *R. sachalinensis*
hybrid P51b

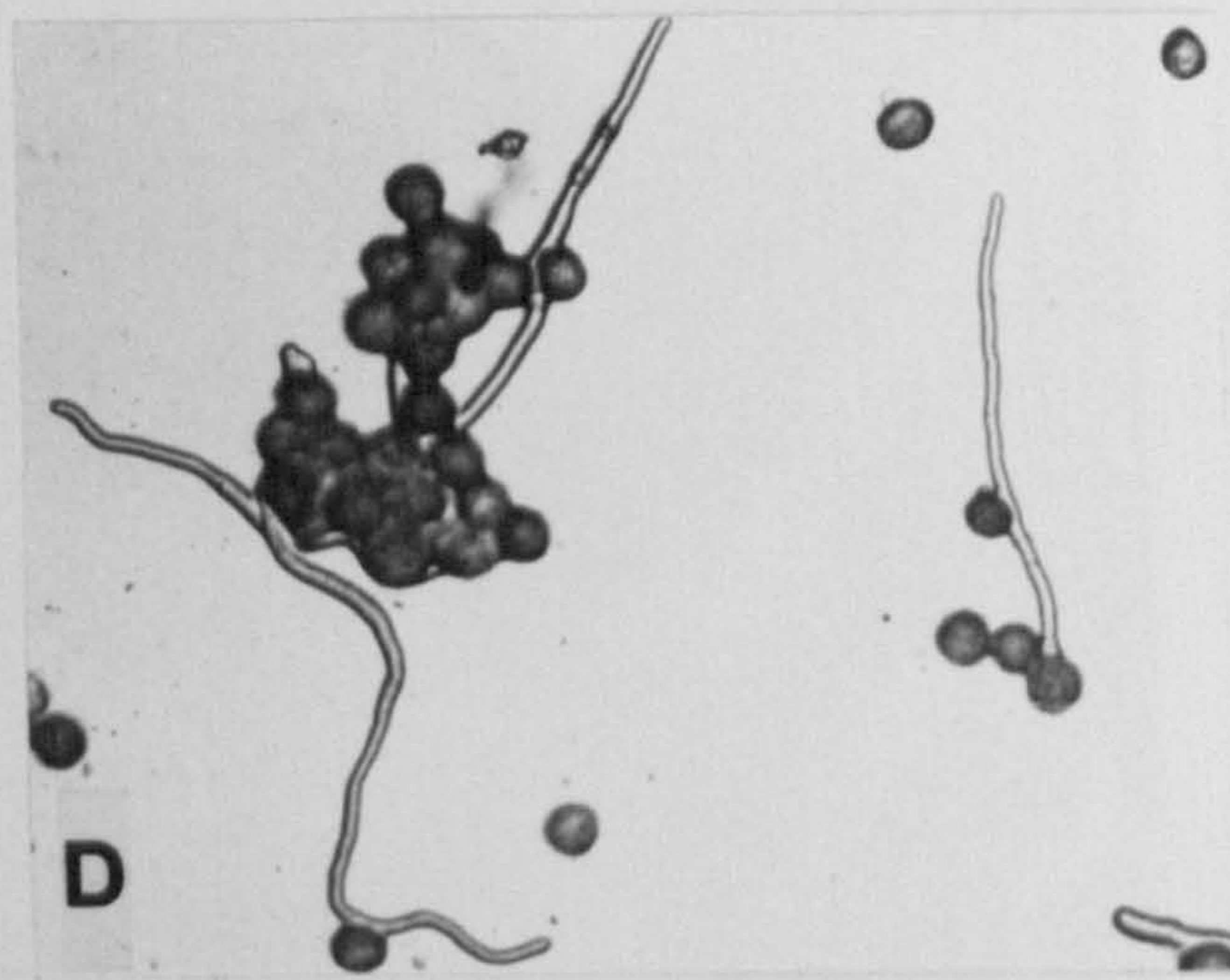
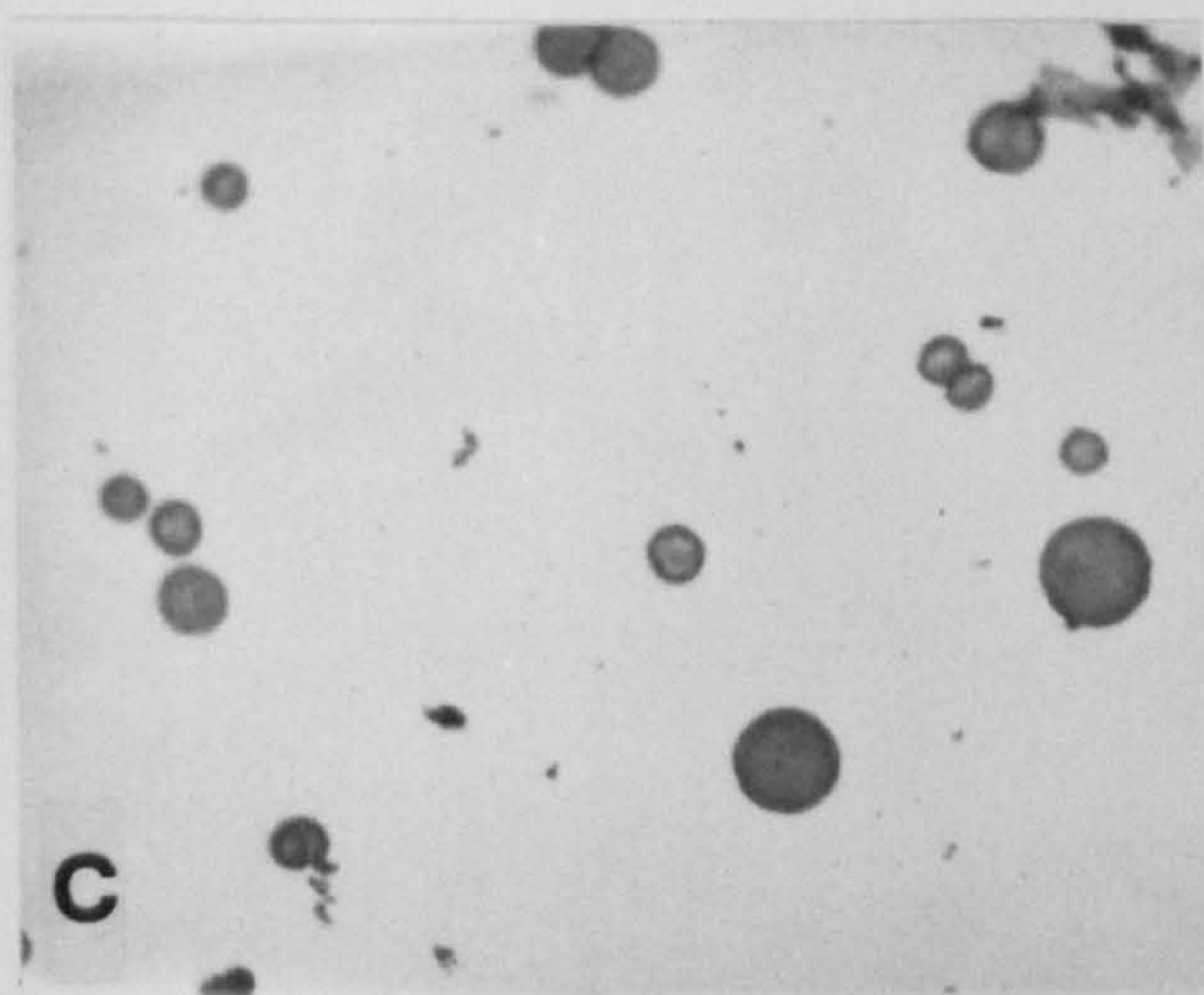
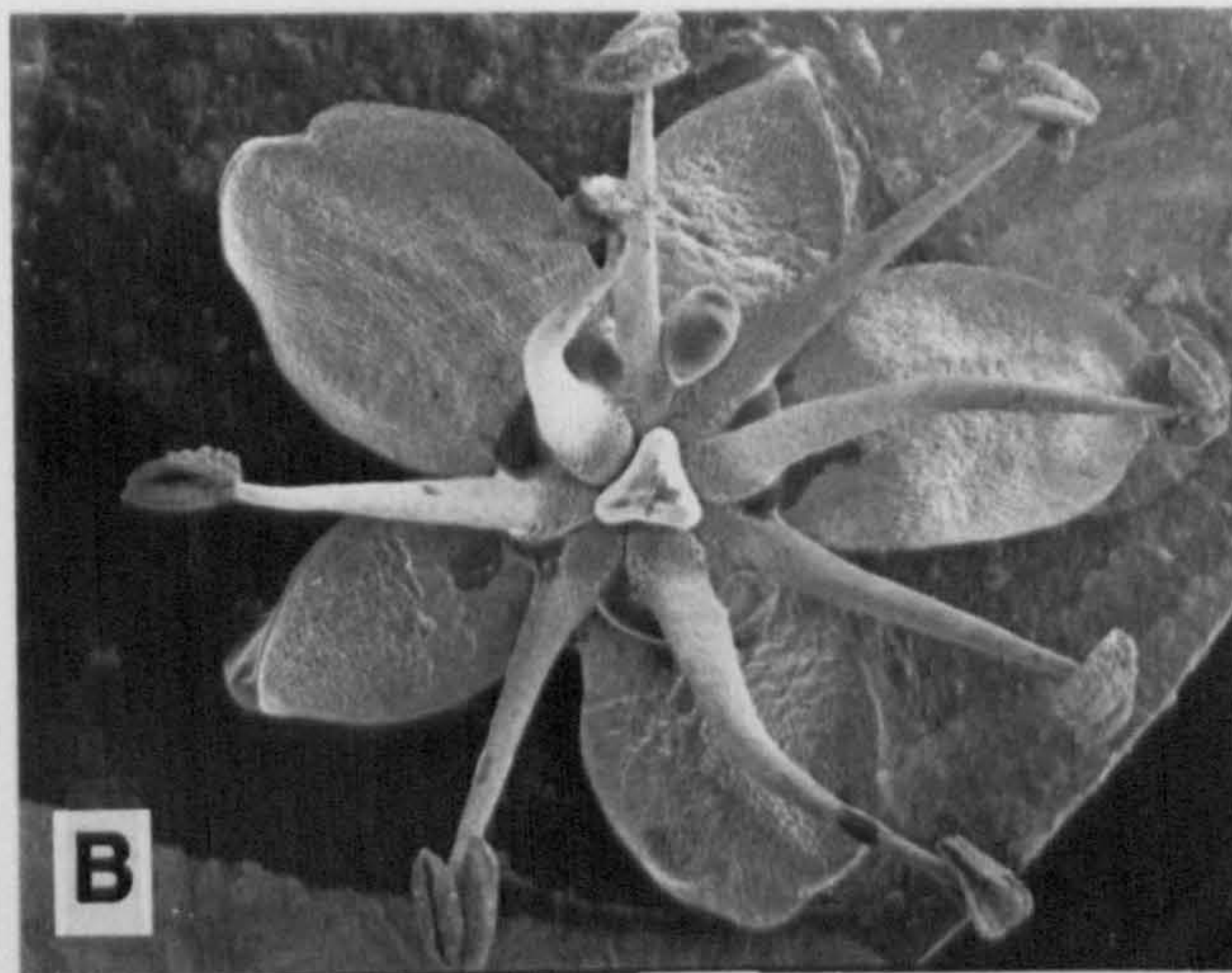
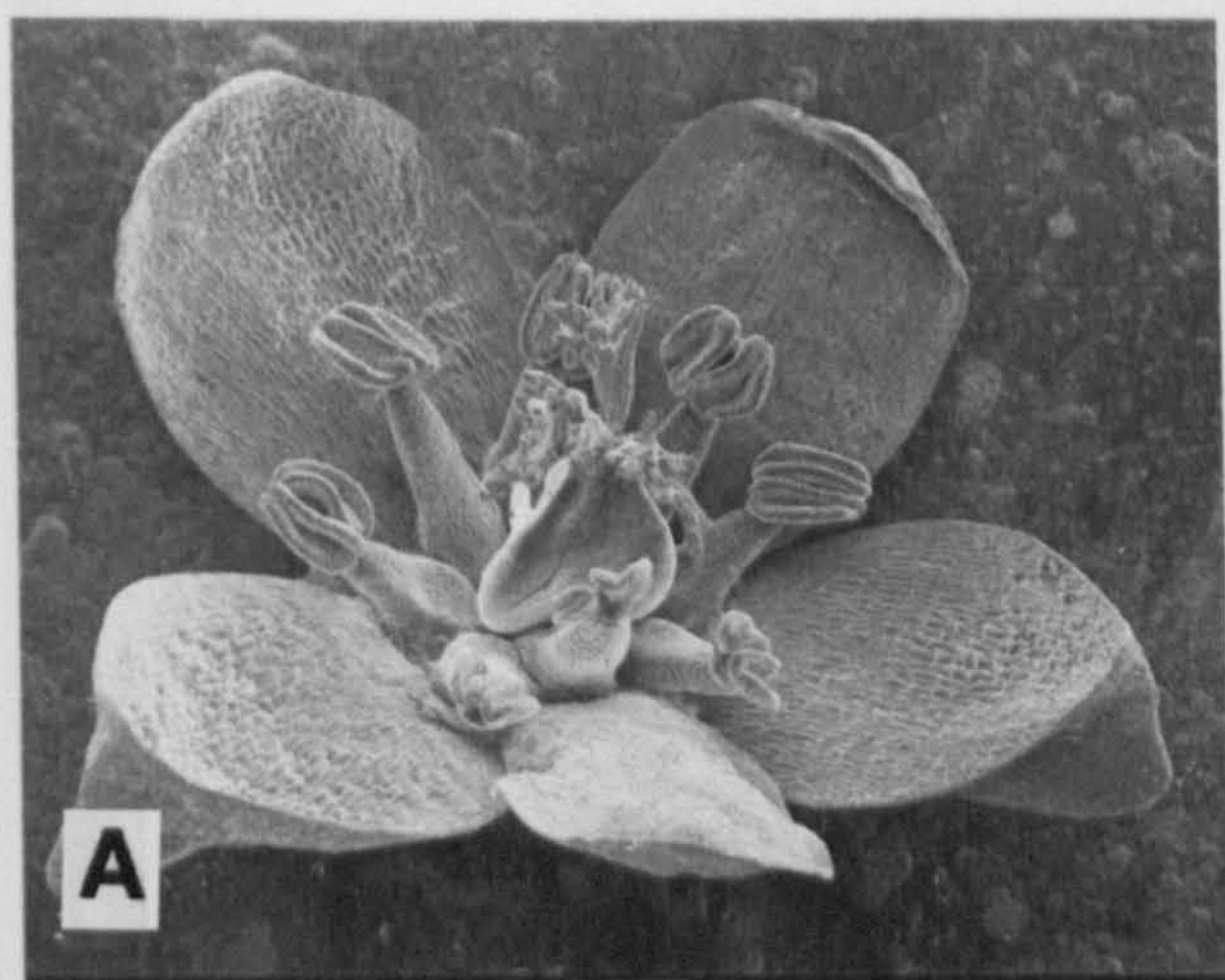


Plate 5. 22

- A** Female artificial *R. japonica* x *R. sachalinensis* hybrid P75c
scale bar 467 microns
- B** Male- fertile plant from the same cross P75d uncoated live flowers
scale bar 680 microns
- C** Artificial *R. sachalinensis* x *F. baldschuanica* P101a pollen X175
- D** Octoploid *R. japonica* x *R. sachalinensis* P51b, showing germinated pollen X 125

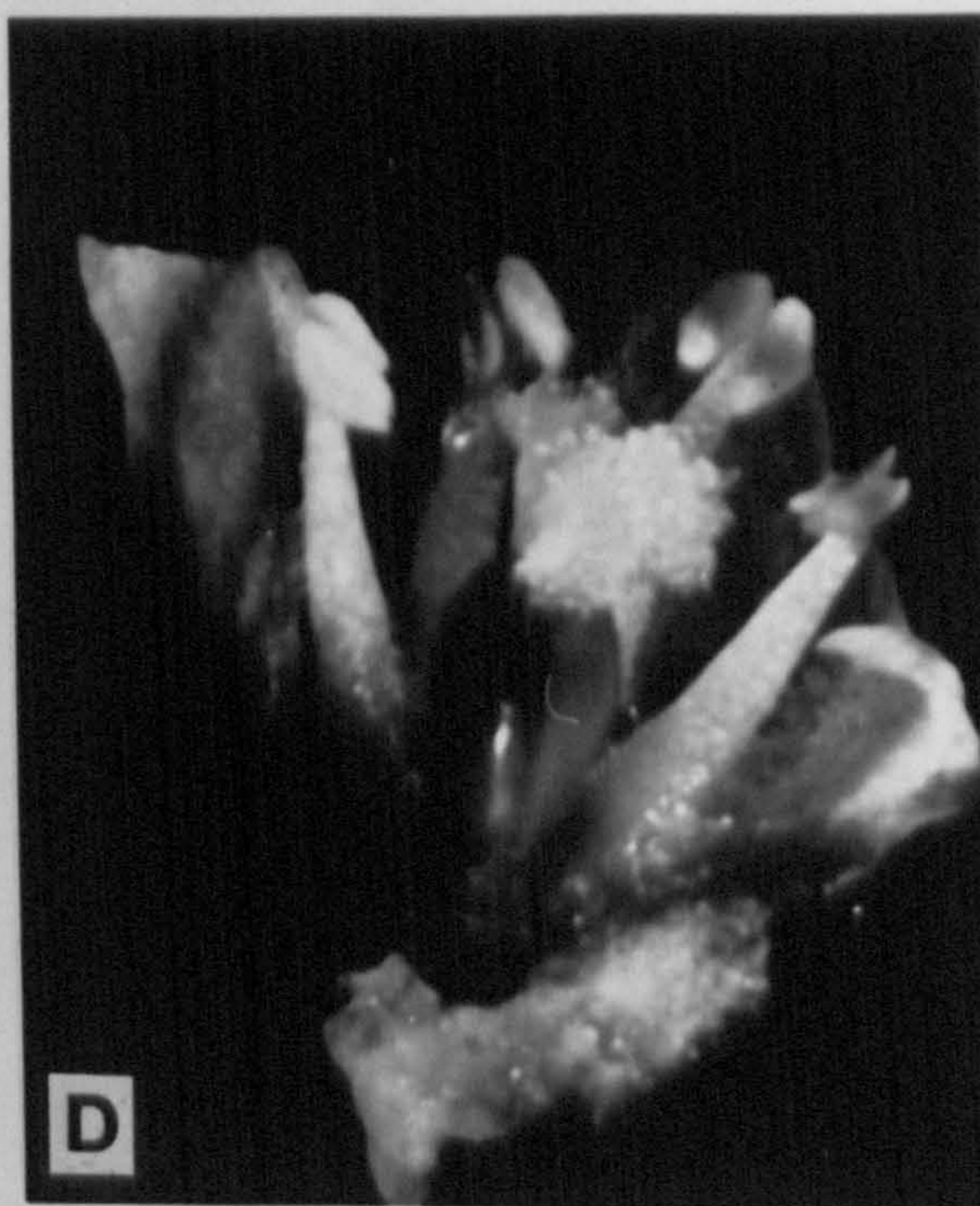
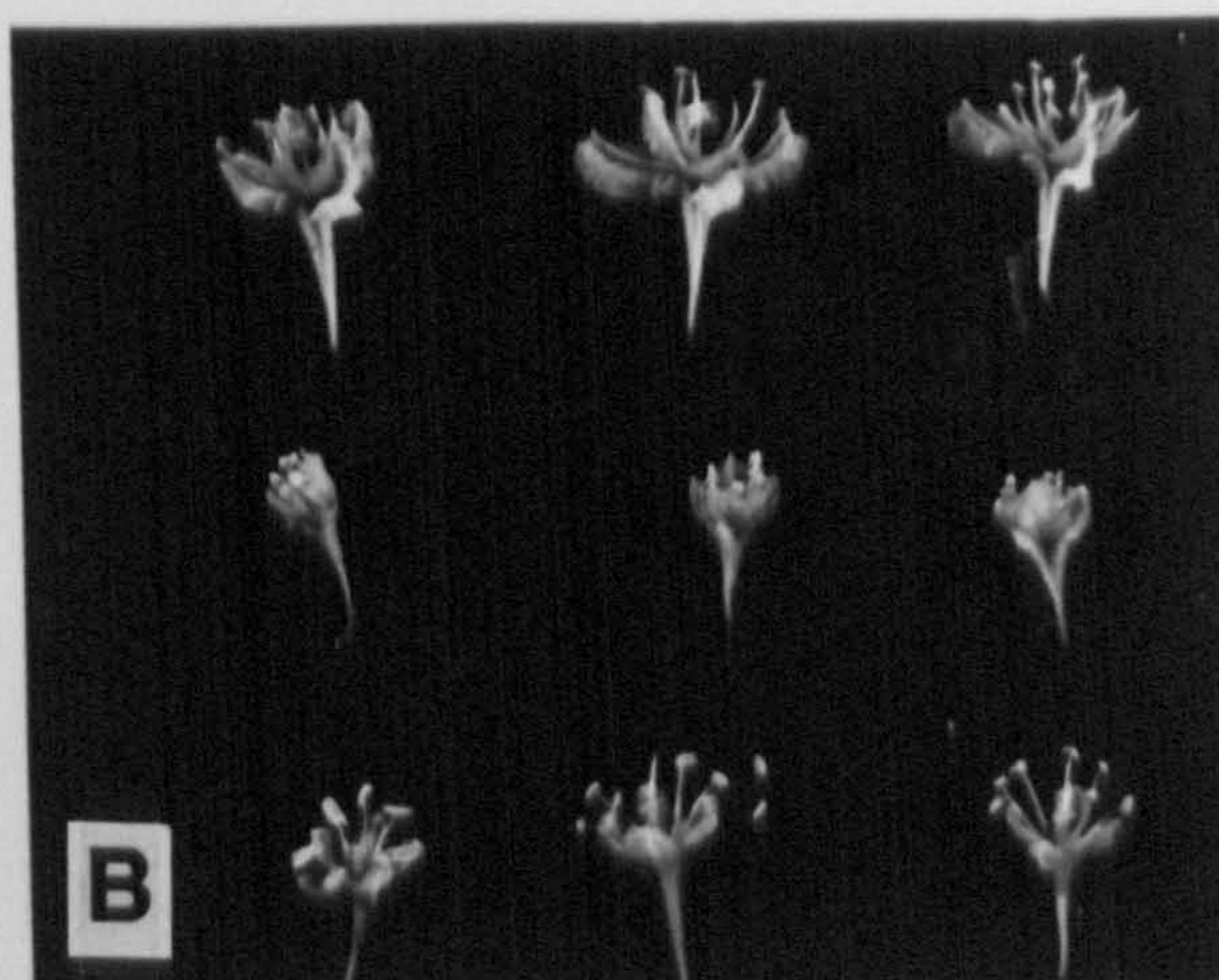


Plate 5. 23 Putative (*R. japonica* X *R. sachalinensis*) X *F.baldschuanica*

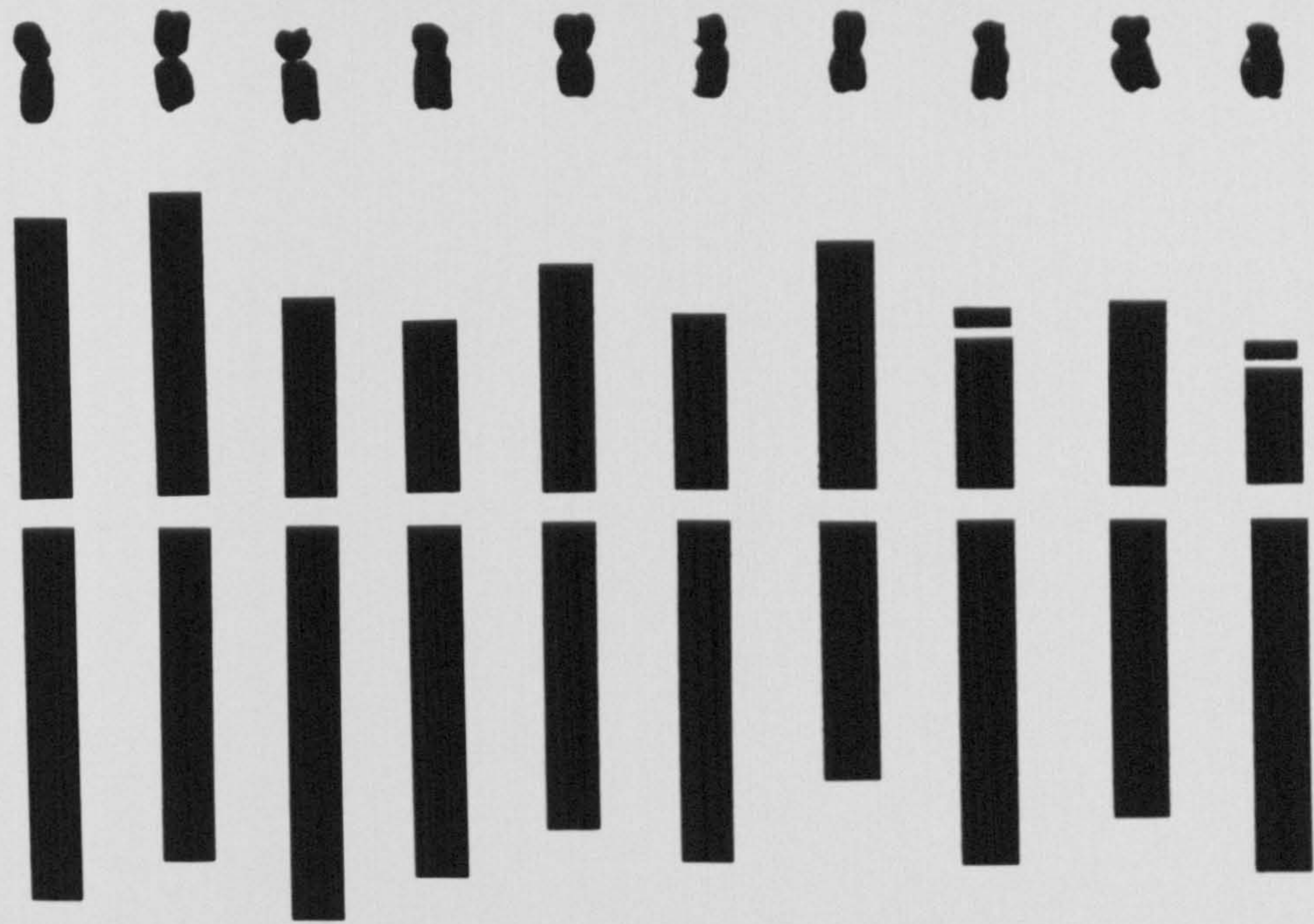
A Inflorescence P 102b X 1.7

B Top to bottom *F. baldshuanica*, putative hybrid P102b, and *R. japonica* P114b

C P102b X 0.4

D P102a flower X 20 to show intermediate morphology of stigma

A



B

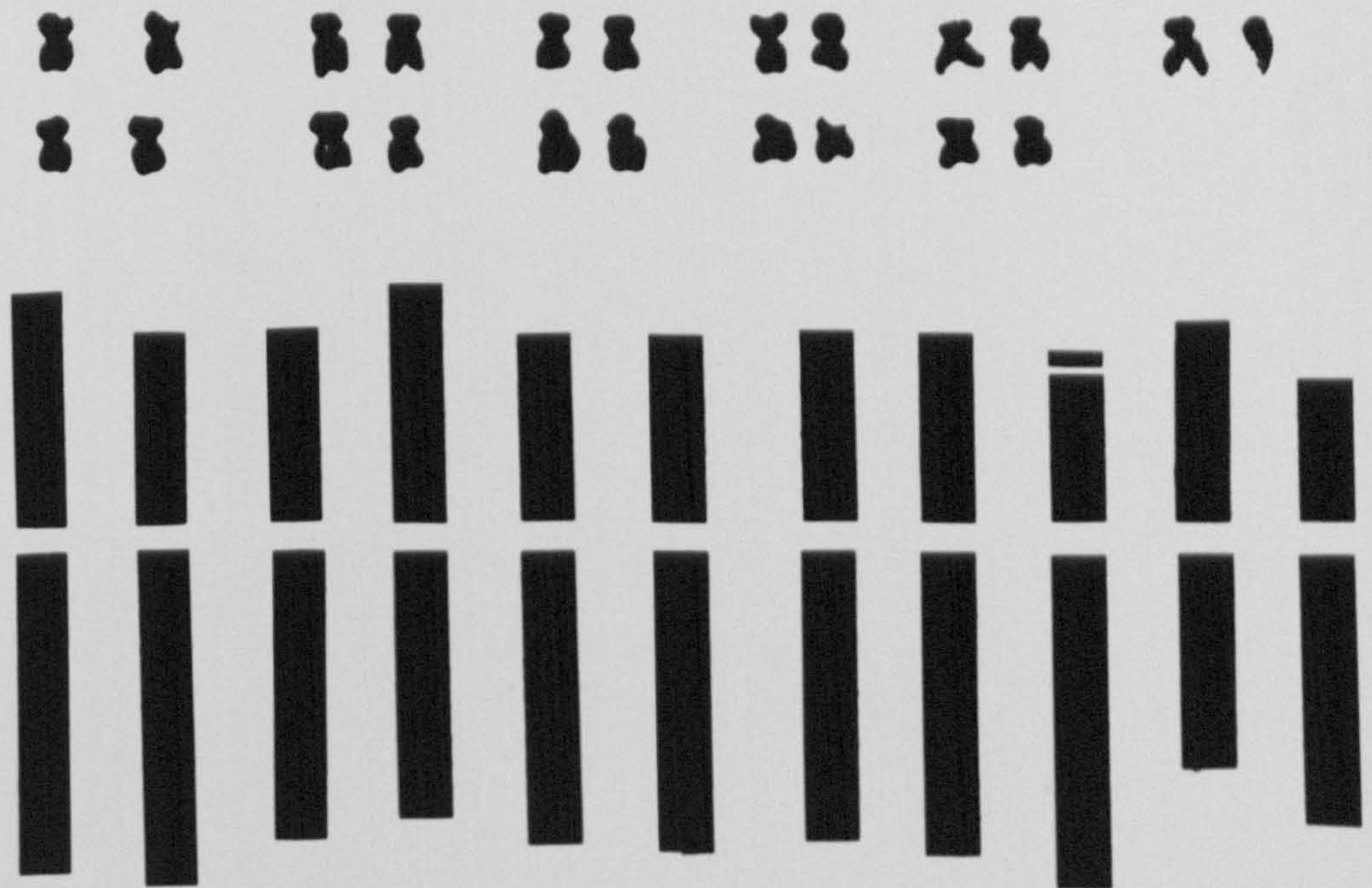


Figure 5. 1 Karyotype and karyogram of artificial *R. sachalinensis* X *F. baldschuanica* hybrid P101b $2n = 32$

A *Fallopia* complement

B *Reynoutria* complement

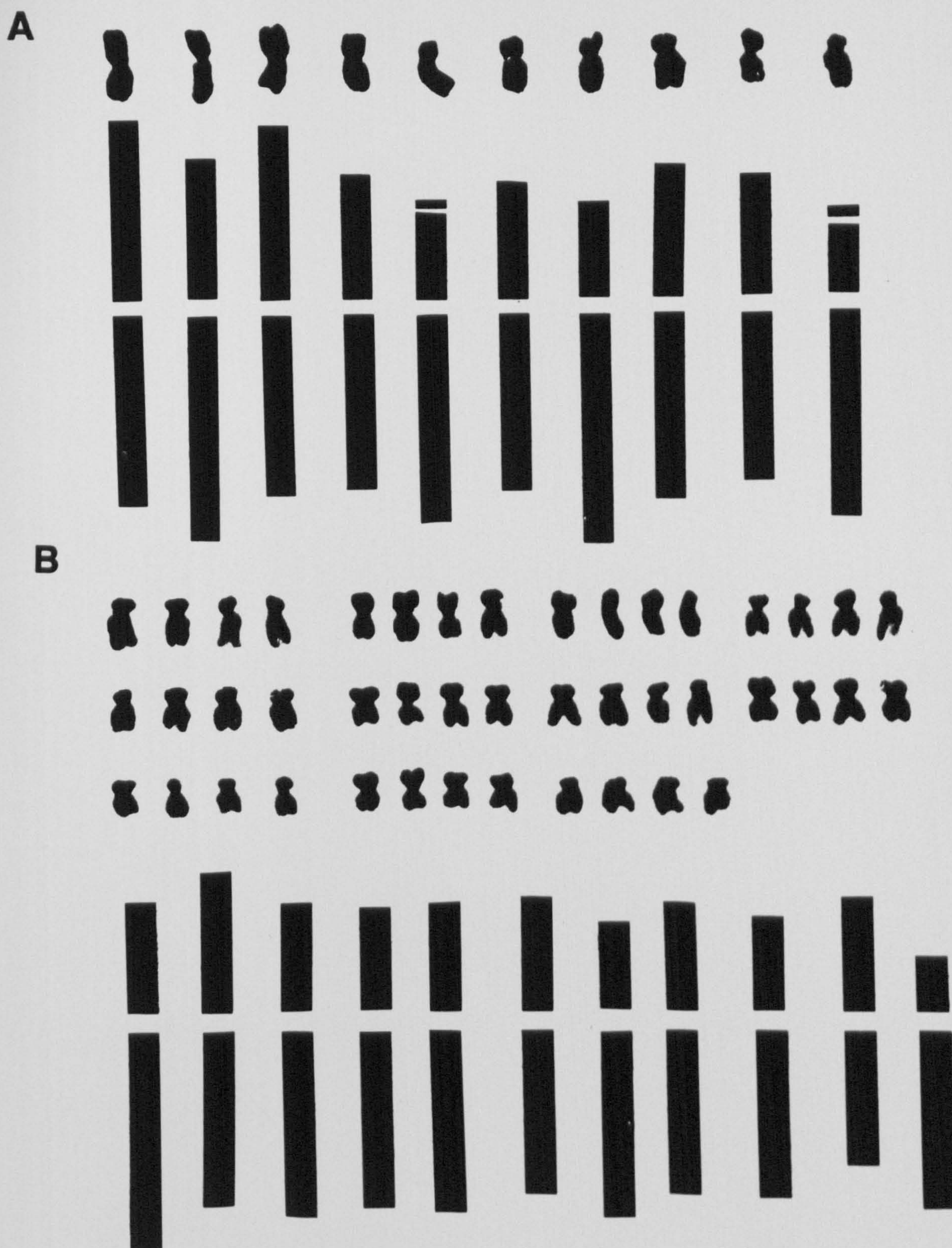


Figure 5.2 Karyotype and Karyogram of putative *R. japonica* X *F. baldschuanica* hybrid P91 $2n = 54$

A *Fallopi* complement

B *Reynoutria* complement

Chapter 6

Synonymy and nomenclature

6.1 INTRODUCTION

In this Chapter the classification of the Family and in particular that of Polygonum s. lat. is dealt with in some detail, as are the various treatments of taxa within section Tiniaria Meissner. The history of the plants in Britain is also discussed and a comprehensive synonymy presented. The classification of the genera Reynoutria and Fallopia is discussed in the light of the findings of my research and the recent work of Ronse de Craene and Akeroyd.

For reasons of clarity in this Chapter I will be using the specific combinations under Polygonum in ^{the discussion.} Apart from R. japonica which thus becomes P. cuspidatum all the other taxa are readily identifiable, and differ only in the endings of the specific epithets.

6.2 THE HISTORY OF REYNOUTRIA AND FALLOPIA INTRODUCTIONS TO BRITAIN

The date of introduction to this country of R. japonica is often given as 1825 (Conolly 1977, Salisbury 1964). There is, one might think, nothing very controversial or unusual about this; after all the plant was first described in 1777. However although the genus Reynoutria and its first species were described by Houttuyn in 1777, together with a perfectly acceptable illustration, the first edition of *Index Kewensis* (1895) described it as *Inc. Sedis* (of uncertain position). It was not until 1901 that Makino recognised that the Reynoutria of Houttuyn was none other than the common P. cuspidatum of Siebold and Zuccarini. Nakai (1926) used Reynoutria at the generic level in a paper in Japanese and Danser (1926) published a German translation together with the original description and illustration in facsimile, whilst Merrill (1938) published details of this in English. This history sheds some light on Moldenke's (1933) choice of generic name (Pleuropterus) being changed shortly afterwards (Moldenke 1941) to Reynoutria (see Table 6.4). By the same token it explains Hedberg's (1946) contention that Tiniaria should be made a 'nomen conservandum' over Reynoutria, as the latter had had little currency in the botanical literature up to that time, whilst the name Tiniaria dated back to Meissner (1826) and had been much used at the sectional level.

Houttuyn's use of the Linnaean system of classification and the fact that the R. japonica he examined had ten stamens,

meant that it was separated from the Polygonums in a separate order Decandria trigyna. This, and the fact that Houttuyn published in Dutch, probably contributed to Reynoutria being lost to botanists for more than 120 years. In the mean time R. japonica had been independently discovered and named P. cuspidatum by Siebold and Zuccarini in 1846. Since it is likely that the specimens Thunberg brought back from Japan for Houttuyn were dried, one must look to von Siebold for the introduction of this taxon to Europe.

Siebold was deported from Japan in 1829, and in addition to his works on the flora, fauna, history, geography and language of Japan found time to satisfy the popular demand for oriental plants by founding a nursery at Leiden. Price-lists of new plants from Japan, China and Java from the gardens of von Siebold and Company were fortunately published in the Year books of the "Royal Dutch Society for the encouragement of Horticulture, under the sponsorship of His Majesty King Wilhelm II". An examination of these lists (Kimura Kitamura 1977, von siebold 1848) reveals that whilst a "Polygonum pictum" appears in 1844 with an introduction date of 1841, P. Sieboldii Reinw. (= P. cuspidatum) does not appear until 1848 under the heading 'Plantes nouvellement importées du Japon'. A foot note describes the particular virtues of this plant and further adds that in 1847 it won a gold medal for the most interesting new garden plant at Utrecht. The catalogue advertised 'une plante mère; 25 plantes très fortes' all for the sum of 500 francs; which considering the same catalogue asked 1 to 6 francs for a

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Wisteria sinensis, must have been a considerable sum of money. It is interesting to note that the catalogue uses P. sieboldii rather than the P. cuspidatum that Siebold had described two years previously. Whether this was for reasons of personal vanity or good business must be left to conjecture. De Vriese (1849) stated that Reinwardt recognised the plant as a new species at about the same time as Zuccarini, but felt that it would be best to keep to the already published name given by Zucarini. He accordingly gave P. sieboldii and P. pictum as synonyms of P. cuspidatum. He also figured an accurate illustration of a male-sterile R. japonica which matches the ubiquitous octoploid. The illustration was said to be drawn from life with the consent of von Siebold, the Honary Chairman of the society and the sole owner of this plant species in Europe. P. pictum is described as being an ornamental perennial obtaining a natural height of 60cm. This very much suggests that P. pictum was in fact R. japonica var. compacta, the much less striking dwarf variety; though in the absence of a description, illustration or specimen one cannot be absolutely sure.

Hooker (1880) said of P. cuspidatum that it was introduced many years ago and that it had been cultivated at Kew for a quarter of a century, and he believed it has been sent from Holland, thus rather confirming von Siebold as the source of the introduction. I have thus no idea how the date of 1825 as the date of introduction was arrived at.

By the 1856 price-list a whole paragraph (see Frontispiece)

was dedicated to the plant, including the telling phrase 'une plante d'ornement vivace, inextirpable....' the price for one hundred very strong plants having unaccountably dropped to 25 francs.

The introduction of the other ornamental taxa in this group to Europe was more recent and rather better documented than the case of R. japonica. R. sachalinensis was said by Hooker (1881) to have been in cultivation at Kew for at least twenty years, and it was, he believed, grown from material sent by one of the Kew collectors in Japan, Messrs Oldham or Wilford. Since it had been discovered on Sakhalin Island in 1853 by Maximowicz and described in 1859, it seems that little time elapsed between its discovery and its introduction.

Russian Vine has had at least two separate introductions. Originally discovered in 1883 near Mt. Sevistan in Russia by Albert Regel, who named it Polygonum baldschuanicum, it was, according to Hooker (1897), grown at Kew from seed sent from St. Petersburg and flowered in 1896. In 1899 Louis Henry received seed from western Szechwan China, sent by the missionary Father George Aubert. These plants from the chinese part of the range were named Polygonum aubertii by him in 1907. Bean (1976) states that very little seed is set and that propagation is best from cuttings; this may well explain the uniformity of British plants.

Fallopia multiflora, although relatively hardy, has not been grown much as a garden plant here, and accordingly it is not

necessary to elaborate further.

6.3 CLASSIFICATION OF THE FAMILY

Melchior's classification of the Polygonales is shown in Table 6.1. The Polygonaceae are the sole family. He divided the family into 3 sub-families and 6 tribes. Subfamily Eriogonoideae is restricted to North America and Chile, subfamily Polygonoideae is primarily of Eurasian distribution, whilst subfamily Coccoloboideae is neo-tropical and is not found in Europe or Asia.

Table 6.2 shows a selection of the major classifications of the family above generic rank. Some authors, such as Bentham and Hooker (1880) and Perdrigeat (1900), favoured tribes as their highest infra-familial ranks, whilst others, such as Meissner (1826, 1856), Gross (1913), Jaretzky (1926) and Haraldson (1978), preferred subfamilies. The taxa with which this study is concerned belong to the subfamily Polygonoideae, though their actual position at lower levels and the generic boundaries are subject to somewhat varied treatments. The traditional treatment is to include all these taxa in one or more sections of Polygonum s.lat. within the tribe Polygoneae. Though, Haraldson (1978), in her revision of the sub-family Polygonoideae placed them in the tribe Coccolobeae.

At the generic or sectional level there is little agreement over classification of Polygonum s.lat. Many authors (see Table 6.3) delimit Koenigia and Fagopyrum at the generic level, but apart from these there exists a plethora of different sectional and generic names. The original

sub family Eriogonodeae

tribe Hollisterieae

genus Pterostegia (1) California
Hollisteria (1) California
Nemacaulis (1) California

tribe Eriogoneae

genus Eriogonum (ca. 200) S.W. N. America
Chlorizantho (CA. 50) California, Chile

sub family Polygonoideae

tribe Rumiceae

genus Oxyria (2)
Rumex (200) N. and S. temperate
Rheum (40) China, C. Asia, S. Russia, Syria
Emex (2) S. Africa, Mediterranean

tribe Polygoneae

genus Oxygonum (30) Trop. Africa, S. Africa,
Madagascar
Atraphix (18) W. Asia, S. Russia,
Mediterranean
Calligonum (35) W. Asia, S. Russia,
Mediterranean
Polygonum (s.lat. ca. 200) Cosmopolitan
Fagopyrum (2) N. China, Turkestan, Siberia,
C. Asia.

sub family Coccoloboideae

tribe Triplarideae

genus Triplaris (20) Tropical S. America
Ruprechtia (17) Mexico, S.Am., Trinidad.

tribe Coccolobeae

genus Antigonon (3-4) Mexico, C. Am., W. Africa
Brunnichia (4) N. Am.
Muchlenbeckia (20) Australasia, Mexico, W.
Africa C. Am.
Homalocladium (1) N. California
Coccoloba (125) Neotropical, subtropical.

TABLE 6.1 CLASSIFICATION OF THE POLYGONALES AFTER MELCHIOR
(1964)

Neisner 1856	Eriogonoideae "subordo Eriogoneae"	Pterygocarpaceae Rabarbareae	Rumiceae Atraphaxinaceae	Polygonoidae ("subordo Polygonaceae") Apterocarpaceae Cerato-goneae Koenigia	Coccolobeae	Brunni- chio- ideae	Symme- rioideae
Bentham & Hooker 1883	Eriogoneae	Koeni- gieae	Rumiceae	Polygonaceae Oxygo- num Atra- phaxis	Coccolobeae		Triplareae
Hammer 1893	Rumicoideae Eriogoneae Eriogoninae	Rumiceae Koeni- giinae		Polygonoidae Atraphaxaceae	Coccolobeae	Coccoloboideae	Triplareae
Perdrigat 1900	Rumiceae Koenigia			Calligoneae	Polygonaceae		Muehlenbeckiaceae
Gross 1913b	Eriogonoideae Holliste- ricae	Rumiceae Eriogoneae		Polygonoidae Atra- phaxinaceae	Polygonaceae Polygoninae Koenigia	Coccolobeae Cocco- lobinae	Triplareae Gymno- podinae Tripla- rinae
Jaretzky 1925	Eriogonoideae Holliste- ricae	Rumiceae Eriogoneae		Polygonoidae Atra- phaxinaceae	Polygonaceae Polygoninae Koenigia	Coccolobeae	Triplareae
Present author		Rumiceae		Polygonaceae Persicariaceae	Coccolobeae		Triplareae

TABLE 6.2

MAIN PREVIOUS CLASSIFICATION OF THE POLYGONACEAE AFTER HARALDSON 1978

Linnaeus 1753 1767	Dumortier 1827	Meisner 1826 1856	Benth and Hooker 1883	Hooker 1890	Dawson 1893	Greene 1904	Gross 1913 a,b	Jaretzky 1925	Danser 1927	Steward 1930	Small 1933	Komarov 1936	Hedberg 1946	Nakai 1952	Grintzes- co 1952	Tutin et al. 1964	Hara 1966	Present author
KOEN	-	KOEN	KOEN	KOEN	KOEN	-	KOEN	KOEN	-	KOEN	-	KOEN	KOEN	-	-	KOEN	KOEN	KOEN
ATRAPHAXIS	-	Tephis	Tephis	Ejeuth	Tephis	DURAVIA	-	Tephis	-	-	-	-	Tephis	-	-	-	-	Tephis
-	-	Avic	Avic	Avic	Avic	POLYG	POLYG	AVIC	Avic	Avic	Temia	Avic	Avic	POLYG	Avic	POLYG	POLYG	Duravia
-	-	Ps mol	Ps mol	Ps mol	Ps mol	-	-	-	-	-	-	-	Ps mol	-	-	-	-	POLYG
-	-	Tovara	Tovara	Tovara	Tovara	-	Tovara	PERS	-	Tovara	Tovara	-	Tovara	Tovara	-	-	ANTEN	Tovara
Pers	Pers	Pers	Pers	Pers	Pers	PERS	PERS	PERS	Pers	Pers	PERS	Pers	PERS	PERS	Pers	Pers	PERS	PERS
-	-	Amblyg	-	Amblyg	-	-	-	-	-	-	-	-	-	LAGU- NEA	Amblyg	-	-	-
-	-	Ceph	Ceph	Ceph	Ceph	-	Ceph	-	Ceph	Ceph	-	Ceph	-	AMPEL	Pers	-	-	Ceph
-	-	Echin	Echin	Echin	-	TRAC	Echin	-	-	Echin	-	Echin	-	CHYL	-	-	-	Echin
-	-	Acon	Acon	Acon	Acon	-	Acon	-	Acon	Acon	-	Acon	PLEU- PYRUM	PLEU- PYRUM	Acon	Acon	ACON	ACON
-	-	Bist	Pers	Bist	Pers	BIST	BIST	Bist	-	Bist	-	Bist	BIST	BIST	Bist	Bist	BIST	BIST
-	-	-	Pleu	-	Pleu	-	PLEU	-	-	-	PLEU	Pleu	-	RY	Pleu	REY	-	REY
-	-	-	Tin	Tin	Tin	BILD	-	POLYG	Tin	-	TIN	-	TIN	BILD	Sam	BILD	BILD	Pleu
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	FAL
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Parog
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	FAG
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pterox

TABLE 6.3

SUMMARY OF MORE IMPORTANT CLASSIFICATION OF POLYGONUM S.LAT.
 GENERA ARE PRINTED IN CAPITALS, SECTIONS IN LOWER CASE;
 CONTINUOUS LINES SEPARATE GENERA, BROKEN LINES SECTIONS.
 (AFTER HARALDSON, 1978)

circumscription by Linnaeus into the genera Koenigia and Polygonum, and the division of Polygonum s.lat., is still quite recognisable in subsequent classifications despite the proliferation of sections and the rather piecemeal promotion of some of these to genera. The modern trend seems to be towards making the division at the generic level. Hence Haraldson (1978) recognised eight genera in lieu of Polygonum s.lat., whilst Webb and Chater (1963) recognised Polygonum with four sections and Koenigia, Bilderdykia, Reynoutria and Fagopyrum as separate genera. Lousley and Kent (1981) followed this but replaced Bilderdykia with Fallopia, an older name. Ronse de Craene and Akeroyd (1988) made a number of fundamental changes in their classification of Polygonum s.lat. They divided it into two tribes Persicarieae and Polygoneae, the former comprising the genera Koenigia, Fagopyrum and Harpagocarpus, and sections Persicariaeae Bistorta, Tovara and Aconogonon of genus Persicaria. Their tribe Polygoneae contained Polygonum, Polygonella, Oxygonum, Pteropyrum, Atraphaxis, Calligonum and Fallopia. Table 6.5 shows the correct name at both sectional and generic ranks for the various segregates of Polygonum s.lat., and it is under these sectional headings that this study is arranged. Section Tiniaria Meissn. is taken to include the large herbaceous perennials P. cuspidatum and P. sachalinense which are sometimes given their own section (Reynoutria Houtt.) Nakai or genus (Reynoutria Houtt.)

Author		Basionym		<u>P. pauciflorum</u> Maxim 1866		<u>Bilderdykia</u> <u>filipes</u> Hara 1972		<u>P. cynanchoides</u>		<u>P. convolvulus</u> Linnaeus 1753		<u>P. dentato-</u> <u>alatum</u> F. Schmidt 1859		<u>P. cristatum</u> Engelm + Gray 1845		<u>P. scandens</u> Linnaeus 1753		
Linnaeus	1762									*Helx.				↓		*Helxine		
Rafinesque	1837									HELX.						HELX		
Meissner	1856			↓						Tin.		↓		Tin.		Tin.		
Bentham and Hooker	1880							↓		Tin.								
Gross	1913			FAG.				FAG.								FAG.		
Nakai	1926			BILD.						BILD.		BILD.						
Samuelsson	1929							Tin.										
Steward	1930							Tin.		Tin.						Tin.		
Komarov	1936			Tin.						Tin.		Tin.						
Moldenke	1933																	
-----	1941			---				---		---		---		---		---		
Hedburg	1946							(TIN.)		(TIN.)				(TIN.)				
Webb & Chater	1963									BILD.								
+ Webb	1964																	
Graham & Wood	1965									Tin.				var.		Tinaria		
Shinners	1967									REYN.				var.		var.		
						↓										REYN		
Holub	1970																	
	1974					FALL				FALL		FALL		FALL		FALL		
Haraldson	1978							FALL		FALL						FALL		
								Para.		Fall.						Fall.		
Ronse De Craene										FALL.						FALL.		
& Akeroyd	1988									Fall.						Fall.		

TABLE 6.4 SUMMARY OF THE MAJOR GENERIC AND SECTIONAL

Names in capitals indicate generic rank, those in lower case taxon not described at time of author's work.

	<u>P. dumetorum</u> Linnaeus 1762	<u>P. cilinode</u> Michaux 1803	<u>P. aubertii</u> Henry 1907	<u>P. baldschuanica</u> Regel 1884	<u>P. multiflorum</u> Thunb. 1784	<u>Pleuropterus</u> <u>cilineris</u> Nakai 1914	<u>Pleuropterus</u> <u>hypoleucus</u> Nakai 1926	<u>Reynoutria</u> <u>japonica</u> Houtt 1777	<u>P. sachalinense</u> Schmidt 1859	<u>P. pterocarpum</u> Wall. 1832
	*Helx	↓		↓	↓			↓		↓
	HELX	HELX.			HELX.					
	Tin.	Tin.			Tin.			Tin.	↓	Tin.
	Tin.		↓	↓	Pleur	↓	↓	Pleur	Pleur	
	BILD				PLEUR	PLEUR	PLEUR	REYN.	REYN.	
			Tin.		Tin.	Tin.		Pleur		
	Tin.		Tin.	Tin.	Tin.	var. cilin Tin.		Tin.	Tin.	Tin.
	Tin.			Pleur I	Pleur I			Pleur II	Pleur II	
---	---	---	---	---	PLEUR REYN	PLEUR REYN	---	PLEUR REYN	PLEUR REYN	---
	(TIN)	(TIN)	(TIN)	(TIN)	(TIN)		(TIN)	TIN		(TIN)
	BILD		BILD	BILD				REYN	REYN	
		Tin.						Tin.	Tin.	
	var. REYN	REYN	REYN	REYN				REYN	REYN	
	FALL	FALL	FALL	FALL						FALL
	FALL Fall	FALL Para	FALL Pleur	FALL Pleur	FALL Pleur			REYN	REYN	
			FALL Sarm	FALL Sarm				FALL Reyn	FALL Reyn	

TREATMENTS OF THE TAXA WITHIN POLYGONUM SECT. TINIARIA.

sectional rank. A vertical line through a column indicates

SECTION	GENUS
<u>Polygonum</u>	
(<u>Avicularia</u> Meissner, <u>Centinode</u> D.C.)	<u>Polygonum</u> S.S.
<u>Aconogonon</u> Meissner	<u>Aconogonum</u> Reichb. (<u>Pleuropteropyrum</u> Gross)
<u>Persicaria</u> (L) Meissner (<u>Echinocaulon</u> Meissner, <u>Cephalophilon</u> Meissner)	<u>Persicaria</u> Miller
<u>Bistorta</u> (L.) D. Don	<u>Bistorta</u> Miller
<u>Tiniaria</u> Meissner	<u>Fallopia</u> Adans. (* <u>Bilderdykia</u> Dumort, (<u>Pleuropterus</u> (Turcz.) Benth., <u>Pleuropterus</u> Turcz., <u>Reynoutria</u> (Houtt.) Nakai)
<u>Helxine</u> (L.) D. Don (<u>Fagopyrum</u> (Miller) Meissner)	<u>Fagopyrum</u> Miller

synonyms in brackets, nomenclatural ones with asterisk.

TABLE 6.5 NOMENCLATURE FOR THE DIVISION OF POLYGONUM S.LAT
AT SECTIONAL AND GENERIC RANK

6.3.1 THE TINIARIA GROUP

The taxa with which this study is concerned are the predominantly Asiatic erect herbaceous perennials and the annual and perennial climbers delineated by Steward (1930) as his section Tiniaria. He defined them as being species with unarmed stems, more or less openly paniculate inflorescences, the outer three perianth segments winged and enlarged in fruit (except in P. cynanchoides, P. cilinode and P. convolvulus), and with the faces of the achenes black and more or less roughened. To this are added any taxa not covered by Steward but included by other authors in Pleuropterus, Bilderdykia, Reynoutria or Fallopia at either generic or sectional levels.

Table 6.4 shows the various taxonomic treatments of the species with which this work is concerned. Since the basionyms of these taxa are spread between four different genera, and individual taxa may be in any of eight different generic combinations, all reference to individual taxa will be made under the Polygonum combination. Section Tiniaria as originally described by Meissner (1826) was a rather heterogeneous grouping since it followed the Linnaean precedent in including those plants with recurved prickles (P. sagittatum L., P. artifolium L. and P. perfoliatum L.). In 1856 Meissner transferred these to section Echinocaulon, and listed additionally P. cristatum in a new group for erect herbaceous plants. A glance at Table 6.3 will indicate that most modern classifications do not recognise section Tiniaria but prefer to confer generic rank on one,

two or more of the segregates. The most usual cause of this is the separation of the large erect herbaceous perennials P. cuspidatum and P. sachalinense from the climbing species. Nakai (1926) divided section Tiniaria into no fewer than three distinct genera. The annuals were placed in Bilderdykia, P. multiflorum in Pleuropterun and the erect herbaceous taxa in Reynoutria. Nakai did not examine the woody perennial P. baldschuanicum and it is interesting to speculate whether he would have made it a fourth genus, or used it as a link to join up two of his other ones.

Hedberg (1946), using morphology, chromosome number and his own pollen work, considered that members of section Tiniaria had their own distinctive pollen type and that Tiniaria should be accorded generic rank. He proposed that Tiniaria should be made a 'nomen conservandum' against the older Reynoutria Houtt. 1777 and Bilderdykia Dum. 1827, and accordingly made the combination Tiniaria japonica, leaving Janchen (1950) to make the remaining combinations.

Löve and Löve 1956 stated that, whilst some palynological and morphological data suggested 'generic association of Bilderdykia and Reynoutria', this was strongly contradicted by the available cytological data. This I take to mean the difference in base numbers between Reynoutria (11) and the annual and woody climbers (10). Webb and Chater (1963) viewed the problem from a European stand point, and placed the annual taxa and P. baldschuanicum in Bilderdykia and kept Reynoutria as a separate genus. They appeared a little diffident about the distinction between Reynoutria and

Bilderdykia, pointing out that the two are connected by some twining woody perennials (Russian Vine) from Asia, but concluded that as the chromosome base number of these climbers is 10 they are better assigned to Bilderdykia. Shinnars (1967), following on from Hedberg, considered that, since Tiniaria had not been made a 'nomen conservandum' and any generic segregate that includes P. cuspidatum must use the oldest name, Reynoutria must replace Bilderdykia. Holub (1971), in his analysis of the protologue for Fallopia Adams (1763), was satisfied that Adanson was referring to Polygonum scandens L., so that any generic grouping (other than Polygonum) that contains P. scandens must properly be termed Fallopia.

Haraldson (1978) in a recent revision of the subfamily, maintained the separation of Reynoutria and Fallopia at the generic level. Fallopia was divided into 3 sections, the annuals being in section Fallopia, section Paragonum containing P. cilinode and P. cynanchoides, and the perennial climbers being assigned to section Pleuropterus. Ronse de Craene and Akeroyd (1988) in the latest treatment of Polygonum s.lat. went one stage further than this and combined Reynoutria with Fallopia as sections in the genus Fallopia.

Although there has been much variation in the treatment of section Tiniaria and its segregants at the generic level, there has with very few exceptions been a broad consensus on what taxa actually belonged to it (Table 6.4). However, Gross (1913) and Grintzesco (1952) considered many of these

taxa to be in the genus Fagopyrum and accordingly transferred them. Moreover, Bentham and Hooker (1880) added P. weyrichii F. Schmidt ex Maxim. and Moldenke (1941) added a further 3 taxa generally considered to belong to section Aconogonon to his genus Reynoutria. It is perhaps strange that it has taken 130 years for taxonomists to recognise what Meissner in 1856 found; that these taxa constitute a good natural group and contain a number of characteristics that distinguish them from the other members of Polygonum s.lat.. The question of whether these taxa should be recognised at the generic rather than the sectional level is one that cannot be taken in isolation, since such a recognition affects the status of the other segregates of Polygonum s.lat. One factor contributing to the diverse treatments of this section is that most workers have been concerned with the production of Floras, and the peculiar distribution of these taxa means that the full range of variation is not represented by any one region. Particularly crucial in this respect is P. multiflorum, since this provides a clear link between the erect herbaceous rhizomatous Reynoutria and the climbers, and yet is often missed out in considerations of this section. Whilst Reynoutria and P. baldschuanicum are all introductions to the European flora, P. multiflorum, although hardy enough, has not become established. This is unfortunate, since an examination of Table 6.6 shows that this taxa tends to break down the distinction that has been made in favour of separating Fallopia and Reynoutria. It is herbaceous and has rhizomes, a clear link with the Reynoutrias; it has 22 chromosomes, the same base number as

Haraldson's Classification (1978)	<u>REYNOUTRIA</u>		<u>FALLOPIA</u> Sect. <u>pleuropterus</u>		
	<u>R. sach.</u>	<u>R. jap.</u>	<u>F. baldsch.</u> including <u>F. aubertii</u>	<u>F. mult.</u>	
Annual/Perennial	Perennial	Perennial	Perennial	Perennial	
Woody or Herbaceous	Herbaceous	Herbaceous	Woody	Herbaceous	
Habit	Erect	Erect	Climbing	Climbing + erect form	
Chromosome base Number	11	11	10	11	
Ploidy Level	4x	4x, 8x	2x	2x	
Distribution	Japan, Kuriles Sakhalin Island	Japan China	Russia Afghani- stan Pakistan	Asia	
Pollen type	Tiniaria	Tiniaria	Tiniaria	Tiniaria	
Stigma type	Fimbriate	Fimbriate	Capitate	Fimbriate	
Sex Expression	Gynodioe- cious	Gynodioe- cious	Hermaphr- odite	Dioecious (acc. Ohwi 1965)	
Winged perianth	+	+	+	+	
Rhizome	+	+	-	+	
Extra floral nectaries	+	+	+	+	
Dendricular type tepal vasculature	+	+	+		

TABLE 6.6 SUMMARY OF CHARACTERS THOUGHT TO BE OF TAXONOMIC
REYNOUTRIA

FALLOPIA Sect. <u>Parogonum</u>		FALLOPIA Sect. <u>Fallopia</u>		
<u>F. cynan.</u>	<u>F. cilinodis</u>	<u>F. convol.</u>	<u>F. dumetorum</u>	<u>F. scandens</u>
	Perennial	Annual	Annual	Annual/Pere.
	Herbaceous	Herbaceous	Herbaceous	Herbaceous
Climbing	climbing + erect form	Climbing	Climbing	Climbing
	10, 11	10	10	10
	2x	2x, 4x	2x	2x
Central China	Eastern America	Circumpolar	Eurasia, N. America	Eurasia, N. America
Tiniaria	Tiniaria	Tiniaria	Tiniaria	Tiniaria
	Fimbriate	Capitate	Capitate	Capitate
Hermaphr- odite?	Hermaphr- odite	Hermaphr- odite	Hermaphr- odite	
	-	-	+	+
	±	-	-	
	+	+		+
		+		+

VALUE FOR THE 9 MORE IMPORTANT TAXA OF FALLOPIA AND

Reynoutria; and it has the fimbriate stigma like Reynoutria in contrast to the capitate one of most of the other taxa. On the other hand its habit is that of Fallopia since it is a climber, though it should be noted that erect forms exist. The above facts plus the sympatric geographical distribution are all strongly suggestive that it is either the diploid progenitor of the Reynoutria or that it and they are derived from the same ancestral stock. In spite of the great similarity between this taxon and the Reynoutrias, its failure to flower has meant that I was unable to attempt any artificial cross-pollinations. However, the extreme crossability of male-sterile Reynoutria plants with Russian Vine has provided a final thread of evidence in favour of merging the genera. Although hybridization between taxa in different genera is no reason on its own to amalgamate them, this and the recent work by Ronse de Craene and Akeroyd provide firm evidence for the amalgamation of Fallopia and Reynoutria. I am also of that opinion.

6.4 SYNONYMY

Fallopia dumetorum (L.) Holub, Folia Geobot. Phytotax.,
6(2):176(1971)

Polygonum scandens L. var. B, Sp. Pl.:364(1753)

Polygonum dumetorum L., Sp. Pl., ed.2:522(1762)

Fagopyrum dumetorum (L.) Schreber., Spicil. fl. Lips.:
42(1771)

Fagopyrum membranaceum Moench, Meth:290(1794), fide
Meissner 1826

Bilderdykia dumetorum (L.) Dumort., Flor. belg:18(1827)

Helxine dumetorum Raf., Fl. Tellur., 3:10(1837)

Tiniaria dumetorum (L.) Opiz, Seznam:98(1852), not seen

Tiniaria alata Montandon., Syn. fl. Jura:270(1856) fide
Ind. Kew.

Polygonum alatum Dulac. Fl. Hautes-Pyrénées:169(1867)
fide Ind. Kew

Tiniaria dumetora (L.) Nakai in Mori, Enum. pl. Corea:
136(1922)

Polygonum scandens L. var. dumetorum (L.) Gleason,
Phytologia, 4:23(1952)

Reynoutria scandens var. dumetorum (L.) Shinn., Sida,
3:118(1967)

Fallopia scandens (L.) Holub, Folia Geobot Phytotax.,
6:176(1971)

Polygonum scandens L., Sp. Pl.:364(1753)

Helxine scandens Raf., Fl. Tellur., 3:10(1837) fide Ind.
Kew.

Tiniaria scandens (L.) Small, Fl. s.e. U.S.:382(1903)
fide Ind. Kew.

Bilderdykia scandens (L.) Greene, Leafl. Bot. Obsn Crit., 1:23(1904) fide Ind. Kew.

Fagopyrum scandens (L.) Gross, Bull. Géogr. Bot., 23:22(1913) fide Ind. Kew.

Bilderdykia scandens (L.) Lunnel, in Am. Midl. Nat., 4:304(1916) fide Ind. Kew.

Polygonum scandens var. scandens (L.) Franch. et Sav.
Enum. Pl. Jap., 2:476 (1879) fide steward 1930.

Reynoutria scandens (L.) Shinn., Sida 3:117(1967).

Fallopia scandens var. dentato-alatum (F. Schmidt ex Maxim)
J. Bailey comb. nov.

Polygonum dentato-alatum F. Schmidt ex Maxim., Mém. Acad. Sci. St Petersburg., 9:232(1859).

Fagopyrum scandens var. dentato-alatum (F. Schmidt ex Maxim.) H. Gross, Bull. Géogr. Bot., 23:23(1913) fide Steward 1930.

Tiniaria scandens Nak. var. dentato-alata Nakai in Mori,
Enum. Pl. Korea:137(1922)

Bilderdykia dentato-alata (F. Schmidt) Kitagawa, Rep. Inst. Scient. Res. Manchoukuo, 3(1):179(1939) fide Ind. Kew.

Fallopia dentato-alata (F. Schmidt ex Maxim) Holub,
Folia Geobot Phytotax., 6(2):176(1971).

Polygonum scandens V. Komarov in Act. Hort. Petrop., 22:138, non L., fide Komarov 1936.

Bilderdykia scandens var. dentato-alata (F. Schmidt)

Nakai fide Ohwi Flora of Japan 1965.

Fallopia scandens var. cristata (Engelm. and Gray) J.

Bailey comb. nov.

Polygonum cristatum Engelm. and Gray Bost. J. Nat. Hist.

5:259(1845)

Tiniaria cristata (Engelm. and Gray) Small, Fl. s.e.U.S.

5:382(1903)

Bilderdykia cristata (Engelm. and Gray) Greene, Leafl.

Bot. Obs. Crit., 1:23(1904)

Polygonum scandens var. cristatum (Engelm. and Gray)

Gleason, Phytologia, 4:23(1952).

Reynoutria scandens var. cristata (Engelm. and Gray)

Shinn., sida, 3(2):118(1967).

Fallopia cristata (Engelm. and Gray) Holub, Folia Geobot

Phytotax., 6(2):176(1971).

Fallopia convolvulus (L.) Á. Löve, Taxon, 19(2):300 (1970)

Polygonum convolvulus L., Sp. Pl.:364(1753).

Polygonum volubilie Gilib., Exercit., 2:435(1792) fide

Petruscu 1977.

Fagopyrum volubile Gilib., Exercit., 2:435(1792) fide

Ind. Kew.

Fagopyrum carinatum Moench, Meth. :290(1794) fide

Petrusca 1977.

Polygonum infestum Salisb., Prodr. (1796) fide Petrusca

1977.

Polygonum convolvulaceum Lam., Fl. Fr., 3:239(1805) fide Petrusca 1977.

Bilderdykia convolvulus (L.) Dumort., Fl. belg.:18(1827).

Helxine convolvulus (L.) Raf., Fl. Tellur., (3):94(1836).

Tiniaria convolvulus (L.) Webb et Moq. in Webb and Berth. Phyt. Canar., 3:221(1841) fide Petrusca 1977.

Tiniaria carinata Montandon, Syn. fl. Jura 7(1856).

Polygonum striatum Dulac, Fl. Hautes-Pyrénées:169 (1867) fide Petrusca 1977.

Polygonum convolvuliforme St. Lager, Ann. Soc. Bot. Lyon, 7:132(1880) fide Petrusca 1977.

Fagopyrum convolvulus (L.) H. Gross, Bull. Geog. Bot., 23:21(1913).

Tiniaria convolvula (L.) Nakai in Mori Enum. Pl. Corea :135(1922).

Reynoutria convolvulus (L.) Shinn., Sida, 3:117(1967).

*Fallopia convolvulus (L.) Holub, Folia Geobot. Phytotax., 6:176(1971).

* Published as a comb. nova by Holub, but with a foot-note indicating F. convolvulus (L.) A. Löve to be the correct name.

Hybrids.

F. convolvulus x F. dumetorum = F. x convolvuloides

(Brugger) Holub, Folia Geobot. Phytotax., 6(2):176(1971)
Polygonum x convolvuloides Bruegger, Jahresber. Naturf.
Ges. Graubünden, Chur, 29(1884-1885:147(1886)) fide
 Holub 1971.

Bilderdykia x convolvuloides (Bruegger) Janchen, Cat.
Fl. Austr., I. Pteridophyt. and Anthophyt., 4:912(1960)
 fide Ind. Kew.

Fallopia multiflora (Thunb.) Haraldson, Acta Univ Ups. Symb
Bot. Ups., 22(2):77(1978).

Polygonum chinense 'Japansch' Houtt., Nat. hist., 8:479,
 t.49 f.3(1777), non P. chinense L.

Polygonum multiflorum Thunb., Fl. jap.:169(1784)

Helxine multiflorum (Thunb.) raf., Fl. Tellur.,
 3:10(1836).

1. Pleuropterus cordatus Turcz., Bull. Soc. Nat. Mosc
 21(1):187(1845) fide Meissner 1856.

Polygonum convolvulus Thunb. ex Mat 59(1912), in syn.
 fide Steward 1930.

Pleuropterus multiflorus (Thunb.) turcz. ex Nakai Feddes
Rep., 13:267(1914) fide Steward 1930.

Reynoutria multiflora (Thunb.) Mold., Bull. Torrey Bot.
Club, 68:675(1941).

Fagopyrum multiflorum (Thunb.) Grint. in Sâvulescu, Fl.
Rep. Popul. Române, 1:476(1952) fide Ind. Kew.

2. Bilderdykia multiflora (Thunb.) Roberty and Vaut.,
Boissiera, 10:55(1964).

1. Turcz. subsequently discovered this to be merely a new binomial for P. multiflorum and accordingly corrected it to Pleuropterum multiflorus. fide Moldenke (1933).
2. Incorrectly listed in Index Kewensis as B. multiflora (G. Griseb.) Roberty and Vaut. (1964)

Varieties

Fallopia multiflora Thunb. var. cilinervis (Nakai) J. Bailey
comb. nov.

Pleuropterus cilinervis Nakai, Feddes Rep., 13:267(1914)
Reynoutria cilinervis (Nakai) Mold., Bull. Torrey Bot. Club, 68:675(1941).

Fallopia cilinodis (Michaux) Holub ex Haraldson, Acta Univ. Ups. Symb. Bot. Ups., 22(2):78(1978)

Polygonum cilinode Michaux, Fl. Bor. Am., 1:241(1803).
Helxine cilinodis (Michaux) Raf., Fl. Tellur., 3:10(1837) fide Ind. Kew.

Tiniaria cilinodis (Michaux) Small, Fl. s.e.U.S.:382,(1903) fide Shinnors 1967.

Bilderdykia cilinodis (Michaux) Greene, Leaflet Bot. Obs and Crit., 1:23(1904) fide Shinnors 1967.

Reynoutria cilinodis (Michaux) Shinn., Sida, 3:117(1967).

Fallopia cilinode (sic) (Michaux) Holub, Folia Geobot. Phytotax., 6:176(1971).

Fallopia cynanchoides (Hemsley) Haraldson, Acta Univ. Ups.
Symb. Bot. Ups., 22(2):78(1978)

Polygonum cynanchoides Hemsley, J. Linn. Soc.,
26:338(1891).

Fagopyrum cynanchoides (Hemsley) H. Gross, Bull. Géog.
Bot., 23:21(1913) fide Steward 1930.

Fallopia baldschuanica (Regel) Holub, Folia Geobot.
Phytotax., 6(2):176(1971).

Polygonum baldschuanicum Regel, Act. Hort. Petrop.,
8:684, pl.10 (1884).

Reynoutria baldschuanica (Regel) Mold., Bull. Torrey.
bot. Club, 68:675(1941).

Tiniaria baldschuanica (Regel) Hedb. ex Janchen, Phyton,
2:75(1950)

Bilderdykia baldschuanica (Regel) Webb, Feddes Rep.,
68:188(1963).

Fallopia baldschuanica (Regel) Holub var. aubertii (Henry)
J. Bailey comb. nov.

Polygonum aubertii ['auberti'] L. Henry, Rev. Hort.,
7:82-3(1907).

Bilderdykia aubertii (L. Henry) Mold. Rev. Sud. Bot.,
6:29(1939).

Reynoutria aubertii (L. Henry) Mold., Bull. Torrey bot.
Club, 68:675(1941).

Tiniaria aubertii (L. Henry) ex Janchen, Phyton,

2:76(1950).

Fallopia aubertii (L. Henry) Holub, Folia Geobot.
Phytotax., 6(2):176(1971).

Fallopia sachalinensis (F. Schmidt ex Maxim) Ronse Decraene,
Bot. J. Linn Soc., 98:369(1988).

Polygonum sachalinense F. Schmidt ex Maxim., Mém. Acad.
Sci. St. Petersb., 9:233(1859) (Prim. fl. amur.).

Reynoutria sachalinensis (F. Schmidt ex Maxim.) Nakai,
Veg. Dagelet Is. :18(1919) fide Steward 1930.

Pleuropterus sachalinensis (F. Schmidt ex Maxim) Mold.,
Bull. Torrey bot. Club, 60:57(1933).

Reynoutria brachyphylla (Honda) Nakai fide Ohwi, Fl.
Jap.:413 1965.

Tiniaria sachalinensis (F. Schmidt ex Maxim.) Janchen,
Phyton, 2:75(1950).

Fallopia japonica (Houtt.) Ronse Decraene, Bot. J. Linn.
Soc., 98:369(1988).

Reynoutria japonica Houtt., Nat. Hist., 2(8):640, t.51,
f.1. (1777).

Polygonum cuspidatum Siebold and Zucc., Abh. Akad.
Muench., 4(2):208 1846 (Fl. Jap. Fam. Nat., 2:84).

Polygonum pictum Siebold, Jaarb. Nederl. Maatsch. Aanm.
Tuinb., :35(1844) Nom. nud.

Polygonum sieboldii Reinw. fide De Vriese, Jaarb.
Nederl. Maatsch. Aanm Tuinb.:31(1849).

Polygonum forbesii Hance, J. Bot. Lond., 21:100(1883).

fide Steward 1930.

Polygonum zuccarinii Small, Mem. Bot. Col. Coll., 1:158.

t.68. (1895) fide Steward 1930.

Polygonum Reynoutria (Houtt.) Makino, Bot. Mag. Tokyo., 15:84(1901).

Pleuropterus Zuccarinii (Small) Small, Ill. fl. n. U.S., ed 2, 1:676, f.1655(1913) fide Steward 1930.

Pleuropterus cuspidatus (Siebold and Zucc.) H. Gross, Beih. bot. Zbl., 37(2):114(1919).

Reynoutria Henryi Nakai, Sasaski, List Pl. Form. :171(1928) nom nud. fide Steward 1930.

Tiniaria japonica (Houtt.) Hedb., Svensk. bot. Tidskr., 40:399(1946).

Polygonum confertum Hook. f. (fide Ohwi, Fl. Japan 1965)

Reynoutria yabeana Honda fide Ohwi, Fl. Japan 1965

Reynoutria uzenensis (Honda) Honda fide Ohwi, Fl. Japan 1965.

Reynoutria hastata Nakai fide Ohwi, Fl. Japan 1965.

Varieties of F. japonica

Fallopia japonica var. compacta (Houtt.) J. Bailey Watsonia, 17: in press (1989).

Polygonum compactum Hook., f., Bot. Mag., t.6476 (1880).

Reynoutria japonica var. compacta (Hook., f.) Mold., Bull. Torrey Bot. Club, 68:675(1941).

(Reynoutria japonica Houtt. var. compacta (Hook. f.) Buchheim in Zander, Handw.-Buch Bot. Pfl. Namem :744(1972) fide Index Kewensis).

P. sieboldii var. compactum (Hook., f.) Bailey fide Ohwi
Fl. Japan. (1965).

Other Fallopia japonica varieties not fully investigated:

1. Reynoutria elata Nakai Cat. Sem. Hort. Tokyo:12(1914)
 nom. nud. fide Steward 1930.
R. japonica var. elata Nakai fide Steward 1930.
2. Reynoutria japonica var. uzenensis Honda, Bot. Mag., Tokyo, 46:675(1932) fide Honda 1935.
R. uzenensis (Honda) Honda, Bot. Mag. Tokyo, 49:791(1935).
3. Polygonum cuspidatum var. spectabilis de Noter, Rev. Hort. Belg., 35:232(1909).
Reynoutria japonica var. spectabilis (de Noter) Mold., Bull. Torrey bot. Club, 68:675(1941).
4. Reynoutria japonica var. terminalis Honda, fide Ohwi,
Fl. Japan (1965)
5. Reynoutria hachidyoensis var. terminalis Honda, fide
 Ohwi, Fl. Jap. (1965).
6. Reynoutria henryi Nakai, Rigakki, 24:294(1926) fide
 Migo, J. Shanghai. Sci. Inst., sectIII(3):92(1935).
7. Reynoutria yabeana Honda, Bot. Mag. Tokyo, 46:675(1932).
8. Polygonum yunnanense A. Léveillé, Feddes. Rep.,
 6:211(1908).
Reynoutria yunnanensis (A. Léveillé) Nakai ex Migo,
 fide Haraldson 1978.
9. Polygonum Forbesii Hance, J. Bot. Lond., 21:100(1883).

I have received seed from Japan of plants bearing the

specific or varietal names of elata, uzenensis and terminalis. Whilst these have not been planted out long enough to reach maturity, they have not shown enough new variation to warrant treatment as anything other than F. japonica var. japonica or var. compacta.

The taxa represented by the epithets forbesii, yunnanense and henryi are an altogether more interesting case. These are all based on Chinese material and are characterised by a more elliptically shaped leaf, lacking the normal truncate base of F. japonica. A case might be made for varietal status, but would involve a study of the range of variation of live Chinese material. Herbarium specimens are of limited use as the basal leaves are not always collected and are sometimes very different from those on the flowering stems.

F. japonica x F. sachalinensis hybrids

Fallopia x bohemica (Chrtek and Chrtková) J. Bailey
Watsonia 17: in press (1989).

Reynoutria x bohemica Chrtek and Chrtková, J. Nat. Mús. Praha Hist. nat. 152:120(1983).

Reynoutria x vivax Schmitz, J., and Strank, K.J., Gött. Flor. Rundbr., 19(1):19(1985).

The latter is invalid as the authors' included both the Reynoutria and Polygonum combinations in their type

descriptions. In any case I have been shown their type locality and their Reynoutria x vivax is a male-fertile R. sachalinensis plant.

APPENDIX 1

Collection Number	Species	Location/origin	Grid Reference	Chromosome	
				Number	
P1	<u>R. japonica</u> x <u>R. sachalinensis</u>	Roundstone, W. Galway	02.726.424	66	
P2a	<u>R. japonica</u> var. <u>compacta</u>	Bracken Hill (Garden)	51/616.572	44	
P2b	<u>R. japonica</u> var. <u>compacta</u>	Bracken Hill	51/616.572	44	
P3	<u>R. japonica</u>	Ironbridge, Salop	33.671.033		
P4	<u>R. japonica</u> x <u>R. sachalinensis</u>	Brithdir, (Garden) Mer.	23/763.177	66	
P5	<u>R. japonica</u>	Brithdir, (Garden) Mer.	23/761.177	88	
P6	<u>R. japonica</u>	Stoughton, Leics.	43/644.026	88	
P7	<u>R. japonica</u> var. <u>compacta</u>	Broughton Astley, Garden	42/525.927	44	
P8	<u>R. japonica</u>	Criccieth Caerns.	23/492.381	88	
P9	<u>R. japonica</u>	Boston Lodge, Mer.	23/589.382	88	
P10	<u>R. japonica</u>	Pentre'r-felin, Caerns.	23/526.396	88	
P11	<u>R. japonica</u>	Llangwnadl, Caerns.	23/218.335	c88	
P12	<u>R. japonica</u>	Race Course, Leics.	43/617.013	88	
P13	<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>	South Wylam, Durh.	45/124.646	44	
P14	<u>R. japonica</u>	Southmead House Leics.	43.617.015	88	
P15	<u>R. japonica</u>	Stroud, S. Hants.	41/720.234	88	
P16	<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>	A429 Cirencester, E. Glos.	42/039.033	44	
P17	<u>R. japonica</u> x <u>R. sachalinensis</u>	A429 Cirencester, E. Glos.	42/039.033	C66	

P18	<u>R. japonica</u>	Petersfield, S. Hants.	41/744.234	88
P19	<u>R. japonica</u>	Pwllheli, Caerns.	23/374.350	88
P20	<u>R. japonica</u>	Dunton Bassett, Leics.	42/549.892	88
P21	<u>R. japonica</u>	Hindhead, Surrey	41/886.356	88
P22	<u>R. japonica</u>	Chilworth, Surrey	51/012.466	88
P23	<u>R. japonica</u>	Heckfield, Surrey	41/726.612	88
P24	<u>R. japonica</u>	Aberystwyth, Cards.	22/601.820	88
P25	<u>R. japonica</u>	Sibley Leics.	43/602.153	88
P26	<u>R. japonica</u>	Itchen Abbas, N. Hants.	41/541.329	88
P27	<u>R. japonica</u>	Ynys, Merion.	23/597.353	88
P28	<u>R. japonica</u> x <u>R. sachalinensis</u>	Loughborough Leics.	43/544.204	66
P29	<u>R. japonica</u> x <u>R. sachalinensis</u>	Preston	34/510.298	C66
P30	<u>R. japonica</u> x <u>R. sachalinensis</u>	Pont Rhyd Sarn, Merion.	23/859.287	
P31	<u>R. japonica</u> x <u>R. sachalinensis</u>	Maam, W. Galway	02/963.533	66
P32	<u>R. japonica</u> x <u>R. sachalinensis</u>	Lye Green, E. Sussex	51/511.336	C66
P33	<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>	Gomshall, Surrey	51/09 .48	44
P34	<u>R. japonica</u>	Ammanford, Carns.	22/61 .11	88
P35	<u>R. japonica</u>	Tyn Coed, Mer.	23/67 .18	88
P36	<u>R. japonica</u>	Fovey Turn, E. Cornwall	20/052.532	C88
P37	<u>R. japonica</u>	Doublebois turn, E. Cornwall	20/188.643	88
P38	<u>R. japonica</u>	Clevedon Bristol	31/39 .71	
P39	<u>R. japonica</u>	Toxteth, S. Lancs.		88
P40	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cirencester, E. Glos.	42/025.023	66
P41	<u>R. japonica</u> x <u>R. sachalinensis</u>	Small wood end, Cheshire	33/806.602	66

P42	<u>R. japonica</u>	Cardingmill Valley Salop.	88
P43	<u>R. japonica</u> x <u>R. sachalinensis</u>	Ironbridge middle, Salop. 33/67 .03	66
P44	<u>R. japonica</u> x <u>R. sachalinensis</u>	Amroth ditch, Pembs. 22/167.071	66
P45	<u>R. japonica</u> x <u>R. sachalinensis</u>	Amroth male, Pembs. 22/167.071	66
P46	<u>R. japonica</u>	Llandrindrod Wells, Rads 32/058.612	88
P47	<u>R. japonica</u> x <u>R. sachalinensis</u>	Llandrindrod Well, Rads 32/.058.612	66
P48	<u>R. japonica</u>	Llanstephan, Carns. 23/354.112	88
P49	<u>R. japonica</u> x <u>R. sachalinensis</u>	Dolgellau 1984 23/711.183	66
		Mer.	
P50	<u>R. japonica</u> x <u>R. sachalinensis</u>	Dolgellau 1984 Mer. 23/711.183	88
P51A	<u>R. japonica</u> x <u>R. sachalinensis</u>	Dolgellau 1984 Mer. 23/711.183	88
P51B	<u>R. japonica</u> x <u>R. sachalinensis</u>	Dolgellau 1982 Mer. 23/711.183	88
P52	<u>R. japonica</u> x <u>R. sachalinensis</u>	Dolgellau 1982 Mer. 23/711.183	66
P53	<u>R. sachalinensis</u>	Ballyconneely, 02/620.446	C44
		W. Galway.	
P54	<u>R. sachalinensis</u>	Brithdir old coll. Mer. 23/761.177	44
P55	<u>R. sachalinensis</u>	Nant-Y-Frith, Flints. 32/265.542	C44
P56	<u>R. sachalinensis</u>	Errislannen, W. Galway. 02/620.495	44
P57	<u>R. sachalinensis</u>	Howey, Radnor. 32/051.587	44
P58	<u>R. sachalinensis</u>	Howey N. 1984, Radnor 32/052.591	44
P59	<u>R. sachalinensis</u>	Howey Vill. Radnor. 32/051.588	44
P60	<u>R. sachalinensis</u>	Elstead, Surrey 41/98 .41	44
P61	<u>R. sachalinensis</u>	Godalming, Surrey	44
P62	<u>R. sachalinensis</u>	Edwinsford, Carns. 22/632.34	
P63	<u>R. sachalinensis</u>	Falcondale, Cards. 22/569.500	44
P64	<u>R. sachalinensis</u>	Cirencester, E. Glos. 42/025.023	44

P65	<u>R. sachalinensis</u>	Llandewi	32/102.680	44
P66	<u>R. sachalinensis</u>	Aber	/477.483	44
P67	<u>R. sachalinensis</u>	Amroth toilets Pembs.	22/171.071	44
P68	<u>R. sachalinensis</u>	Amroth beach, Pembs.	22/166.071	44
P69	<u>R. japonica</u> var. <u>compacta</u> x <u>F. baldschuanica</u>	Artificial hybrid Leicester		32
P70				
P71	<u>R. sachalinensis</u>	Cwm Ystwth	22/79(8).74(3)	44
P72	<u>R. sachalinensis</u>	Ironbridge 1984, Salop	33/670.033	44
P73	<u>R. sachalinensis</u>	Bryn Eithyn	22/582.782	44
P74	<u>R. sachalinensis</u>	Cwrt Newydd	22/493.477	44
P75a,c,d,e	<u>R. japonica</u> x <u>R. sachalinensis</u>	Artificial hybrid	-	66
P76,a,c, d,e,g,h	<u>R. japonica</u> x <u>R. japonica</u> var. <u>compacta</u>	Artificial hybrid	-	66
P77	<u>R. japonica</u> x <u>F. baldschuanica</u>	Seed from P12 Leics.	43/617.013	54/5
P78a	<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>	Artificial hybrid	-	44
P79a,b	<u>R. sachalinensis</u> x <u>R. japonica</u> var. <u>compacta</u>	Artificial hybrid	-	44
P80a,b,c,d	<u>R. japonica</u> x <u>F. baldschuanica</u>	Seed from P8 Caerns.	23/492.381	54
P81	<u>R. japonica</u> x <u>F. baldschuanica</u>	Seed from Loughborough octoploid	43/544.204	54
P82a,d,e	<u>R. japonica</u> x <u>F. baldschuanica</u>	Seed from P9 Mer.	23/589.382	54
P83b,c,d,e	<u>R. japonica</u> x <u>F. baldschuanica</u>	Seed from P19 Caerns.	23/374.350	54
P84	(<u>R. japonica</u> x <u>R. sachalinensis</u>) x 4x <u>Reynoutria</u>	Seed from open pollinated hexaploid from Strasbourg	43/617.015	55

P85a,b	<u>(R. japonica x R. sachalinensis)</u> x 4x <u>Reynoutria</u>	Seed from open pollinated P31 at Leicester	43/617.015	55
P86a,b,c, d,e	<u>R. japonica x F. baldschuanica</u>	Seed from P26 N Hants	41/541.329	54
P87a,b,c, d,e	<u>R. japonica x F. baldschuanica</u>	Seed from P5 Mer.	23/761.177	54
P88a,b	<u>R. japonica x F. baldschuanica</u>	Seed from P35 Mer.	23/67 .18	54
P89b,c,d	<u>R. japonica x F. baldschuanica</u>	Seed from P3 Salop.	33/671.033	54
P90a,b,c,d	<u>R. japonica x F. baldschuanica</u>	Seed from P18 S. Hants.	41/744.234	54
P91a,b,c	<u>R. japonica x F. baldschuanica</u>	Seed from P10 Caerns.	23/526.396	54
P92a,b,c,d	<u>R. japonica x F. baldschuanica</u>	Seed from P25 Leics.	43/602.153	54
P93a,b	<u>R. japonica x F. baldschuanica</u>	Seed from P27 Merion	23/597.353	54
P94a,b,c	<u>R. japonica x F. baldschuanica</u>	Artificial hybrid		54
P95	<u>R. japonica</u>	Llanbedrog	23/331.321	
P96	<u>R. japonica</u>	Milngavie		88
P97	<u>R. japonica</u>	Gomshall		88
P98	<u>F. baldschuanica</u>	Hinckley Road Leicester		
P99	<u>R. japonica var. compacta</u>	North Ledaig		44
P100	<u>F. multiflora</u>	Not known	-	22
P101a,b c,d	<u>R. sachalinensis x F. baldschuanica</u>	Artificial hybrid	-	32
P102a,b,	<u>(R. japonica var. compacta x</u> <u>R. sachalinensis) x F. baldschuanica</u>	Seed from P33 Surrey	51/09 .48	32
P103a,b,c	<u>(R. japonica x R. sachalinensis)</u> x octoploid	Seed from P49 Mer.	23/711.183	75,76,77
P104	Dolgellau octoploid selfed	Seed from P50	23/711.183	88

P105	<u>R. japonica</u> var. <u>'uzenensis'</u>	Seed from Tokyo	-	66,88
P106	<u>R. japonica</u> var. <u>compacta</u> selfed	Seed from P99	-	44
P107				
P108a,b	<u>Reynoutria</u> hybrid	Seed ex P31 Leicester	-	48,57
P109	<u>R. japonica</u> x <u>R. sachalinensis</u>	Polperro	20/226.516	66
P110	<u>R. japonica</u>	Sligo		88
P111	<u>R. japonica</u> x <u>R. sachalinensis</u>	Maam 1985		C66
P112	<u>R. japonica</u>	Lisdoonvarna		88
P113	<u>R. japonica</u>	Seed from Peking	-	88
P114	<u>R. japonica</u>	Seed from Tokyo No. 148	-	44
P115	<u>R. sachalinensis</u>	Seed from Tokyo No. 376	-	44
P116	<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>	Tottenham Marshes	52/352.909	44
P117	<u>'Reynoutria elata'</u>		-	
P118	<u>R. japonica</u> x <u>R. sachalinensis</u>	Seed ex Strasbourg	-	
P119	<u>R. japonica</u> x <u>R. sachalinensis</u>	Brithdir male Mer.	23/761.177	66
P120				
P121	<u>R. japonica</u> x <u>R. sachalinensis</u>	Whitchurch Glam.	31/14 .80	66
P122	<u>R. japonica</u>	Whitchurch Glam.	31/14 .80	88
P123	<u>R. japonica</u>	Bolton S. Lincs.	34/693.082	88
P124	<u>R. japonica</u> x <u>R. sachalinensis</u>	Recess, W. Galway		
P125	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cheshunt A	52/368.028	44
P126	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cheshunt B	52/368.028	44
P127	<u>R. sachalinensis</u>	Cheshunt	52/368.028	44
P128	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cirencester Abbey	42/025.023	66
		Grounds 1985, E. Glos.		

P129	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cirencester Abbey Grounds 42/025.023	
		1985 Tall plant E. Glos.	
P130	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cirencester Abbey Plant 2 42/025.023	C66
P131	<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>	Cirencester layby 4x 41/039.033	44
		1985, E. Glos.	
P132	<u>R. japonica</u> x <u>R. sachalinensis</u>	Cirencester layby 6x 1985 41/039.033	66
P133	<u>R. japonica</u> x <u>R. sachalinensis</u>	Seed No. 3 ex Strasbourg -	
P134a-f	' <u>R. japonica</u> var. <u>terminalis</u> '	Hachijo Islands, Tokyo -	44
P135	<u>R. japonica</u>	Mynytho, below common	88
P136a,b	<u>R. japonica</u> x <u>R. sachalinensis</u>	Honor Oak cemetery 51/354.744	66
P137a,b,c, d,e,k	<u>R. sachalinensis</u>	Seed from <u>R. sachalinensis</u> 22/171.071	44
		Amroth P67. Pembs.	
P137f	<u>R. sachalinensis</u> x (<u>R. japonica</u> x <u>R. sachalinensis</u>)	Seed from Amroth P67 Pembs.	66
P138a-e	<u>R. sachalinensis</u>	Seed from P68 Pembs.	22/166.071
P139	<u>R. sachalinensis</u> x <u>F. baldschuanica</u>	Seed from P58 Radnor	32/052.591
P140	<u>R. sachalinensis</u> x <u>F. baldschuanica</u>	Seed from P160	
P141b,c	<u>R. sachalinensis</u>	Seed from P64 E. Glos.	44
P142a,b	(<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>) selfed	Seed from P131 E. Glos.	44
P143	(<u>R. japonica</u> x <u>R. sachalinensis</u>) selfed	Seed from P132	50-2+
		Cirencester layby E. Glos.	
P144b	Reynoutria hybrid	Seed from P130	49
P145a,d,e,f	<u>R. sachalinensis</u> x <u>F. baldschuanica</u>	Seed ex P127	32
P146b,c,d,e	(<u>R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>) x <u>F. baldschuanica</u>	Seed from Cheshunt hybrid A. P125	32

P147	<u>(R. japonica</u> var. <u>compacta</u> x <u>R. sachalinensis</u>) x <u>F. baldschuanica</u>	Seed from Cheshunt hybrid B, P126	52/368.028	32
P148	<u>F. cilinodis</u>	Seed ex Warsaw		22
P149a,b	<u>F. dumetorum</u>	Seed ex Lund No. 184		20
P150d	<u>F. convolvulus</u>	Seed ex Italy No. 224		40
P151	<u>F. baldschuanica</u>	Cutting ex Cambridge		20
P152	<u>F. baldschuanica</u>	Cutting ex Cambridge		20
P153	<u>F. baldschuanica</u>	Seed ex P152 Cambridge		20
P154	<u>F. convolvulus</u>	Sibley Leics.	43/602.153	40
P155	<u>R. sachalinensis</u>	Llanisloe House	22/253.087	44
P156	<u>F. cilinodis</u>	Seed ex Montreal		22
P157	<u>R. japonica</u> x <u>F. baldschuanica</u>	Merlewood	35/25.17	54
P158	<u>R. japonica</u> x <u>R. sachalinensis</u>	Bristol	31/531.777	66
P159	<u>R. japonica</u> x <u>R. sachalinensis</u>	Bristol	31/531.777	66
P160	<u>R. sachalinensis</u>	Llanishan reservoir Glam.	31/187.813	
P161	<u>Reynoutria</u> hybrid	Wild coll. seed ex P51b		88
P162	<u>F. multiflora</u>	Seed from Peking		22
P163	<u>F. baldschuanica</u>	Sichuan (China) ex A. Leslie		20
P164	<u>R. japonica</u> x <u>F. baldschuanica</u>	Railway Fields Haringay	51/317.882	54
P165	<u>R. japonica</u> x <u>R. sachalinensis</u>	Southport	34/332.178	
P166				
P167	<u>F. cilinodis</u>	Quebec (granite outcrop)		22
P168	<u>F. cilinodis</u>	Quebec (shade form)		22
P169	<u>R. japonica</u>	Salem, USA. male-fertile		88
P170	<u>R. japonica</u>	Salem, USA. male-sterile		

P171	<u>R. sachalinensis</u>	Tochigi Pref. Japan	44
P172	<u>R. japonica</u>	Tochigi Pref. Japan	44
P173	<u>R. japonica</u> var. <u>'compacta'</u>	Kew gardens (Hort)	
P174			
P175	<u>F. baldschuanica</u>	Seedling ex P98	20
P176			
P177	<u>F. dumetorum</u>	Rogate (T. Rich)	
P178	<u>F. convolvulus</u>	Leicester (HR)	n=20
P179	<u>R. japonica</u>	Loughborough (8x)	
P180	<u>R. sachalinensis</u>	Osgathorpe (Leics.)	
P181	<u>R. japonica</u> x <u>R. sachalinensis</u>	Ayr	26/33.21
P182	<u>F. bladschuanica</u>	Bot. Gard. Leicester	
P183	<u>R. japonica</u>	Upperton road Leic.	
P184	<u>R. japonica</u>	Braunstone Gate Leic.	

CHROMOSOME NUMBERS OF SOME ALIEN *REYNOUTRIA* SPECIES IN THE BRITISH ISLES

Reynoutria sachalinensis (F. Schmidt Petrop.) Nakai $2n=44$.

V.c. 17, Surrey: Elmbeamswood, Elstead, GR 41/89.41

V.c. 43, Rads.: Howey, GR 32/051.587

V.c. 48, Merioneth: Brithdir, Caernywch Hall (garden), GR 23/761.177

V.c. 51, Flints.: Nant Y Frith, Bwlchgwyn, GR 32/265.542

V.c. H.16, W. Galway: Ballyconneely, Connemara, GR 02/620.446; Errislannan, Clifden, GR 02/620.495

Reynoutria japonica Houtt. var. *compacta* (J. D. Hook.) Buchheim $2n=44$.

V.c. 16, W. Kent: Platt (garden), GR 51/616.572

V.c. 55, Leics.: Broughton Astley (garden), GR 42/525.927

Reynoutria japonica Houtt. var. *japonica* $2n=88$.

V.c. 2, E. Cornwall: Liskeard, GR 20/188.643; St Austell, GR 20/052.532 (c. 88)

V.c. 11, S. Hants.: Petersfield, GR 41/744.234; Stroud, Petersfield, GR 41/720.234

V.c. 12, N. Hants.: Heckfield, Hook, GR 41/726.612; Itchen Abbas, GR 41/541.329

V.c. 17, Surrey: Hindhead, GR 41/886.356; Chilworth-Shalford, GR 51/012.466

V.c. 44, Carms.: Ammanford, GR 22/61.11

V.c. 46, Cards.: Aberystwyth, GR 22/601.820

V.c. 48, Merioneth: Dolgellau, GR 23/711.823; Tyn Coed, GR 23/67.18; Brithdir, Caernywch Hall (garden), GR 23/761.177; Boston Lodge, Minfford, GR 23/589.382; Llanfihangel-y-Traethau, GR 23/597.353

V.c. 49, Caerns.: Pentre'r-felin, GR 23/526.396; Pwllheli, GR 23/374.350; Criccieth, GR 23/492.381; Llangwnadl, GR 23/218.335

V.c. 55, Leics.: Knighton, GR 43/617.013; University Botanic Garden, Leicester, GR 43/617.015; Sileby, GR 43/602.153; Stoughton, GR 43/644.026; Dunton Bassett, GR 42/549.892

Reynoutria tetraploids $2n=44$.

V.c. 17, Surrey: Gomshall Station, GR 51/09.48

V.c. 33, E. Gloucs.: Cirencester (plant no. 1), GR 41/039.033

V.c. 66, Co. Durham: South Wylam, GR 45/124.646

Reynoutria hexaploids $2n=66$.

V.c. 14, E. Sussex: Lye Green, GR 51/511.336 (c. 66)

V.c. 33, E. Gloucs.: Cirencester (plant no. 2), GR 41/039.033 (c. 66)

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- V.c. 48, Merioneth: Dolgellau, GR 23/711.823; Brithdir, Caerynwch Hall (garden), GR 23/763.177;
 Pont Rhyd Sarn, near. Bala, GR 23/859.287
 V.c. 55, Leics.: Loughborough, GR 43/544.204
 V.c. 60, W. Lancs.: Preston, GR 42/510.298 (c. 66)
 V.c. H.16, W. Galway: Maam, GR 02/963.533; Roundstone, GR 02/726.424

The rhizomatous perennials, *Reynoutria japonica* (*Polygonum cuspidatum* Siebold & Zucc.) and *Reynoutria sachalinensis* (*Polygonum sachalinense* F. Schmidt Petrop.), introduced to the British Isles last century are now firmly established with a well-earned reputation as invasive and persistent weeds (Conolly 1977). Characteristics which allow *R. japonica* to be an early colonist of lava fields in Japan ensure that it is well able to cope with habitats ranging from urban waste land to Welsh hillsides.

The threat posed by *R. japonica* is now recognized in law since it is one of the land plants which it is illegal to introduce into the wild in Britain. It was against this background that we set out to learn more about the mode of spread and reproductive biology of these plants. Since published work (none of it carried out on British plants) revealed counts of $2n=44$, more than 60, and 88 for *R. japonica* var. *japonica*, $2n=44$ for *R. japonica* var. *compacta*, and $2n=44$, c. 66 and 102 for *R. sachalinensis* (Federov 1969; Moore 1973, 1977), we made an examination of the chromosome numbers of British and Irish plants our starting point.

On the basis of evidence to be presented in a later paper, it appears that the base chromosome number for *Reynoutria* is 11. Our results show that three different ploidy levels are present in Britain. All *R. sachalinensis* and *R. japonica* var. *compacta* plants examined so far are represented only at the tetraploid level. *R. japonica* var. *japonica*, on the other hand, is found to be octoploid at the 24 sites in the survey. The octoploid *R. japonica* is the most usually encountered and the 24 locations were taken on an arbitrary basis. The nine hexaploid plants, however, were collected because they differed in some way from the plants usually encountered, and morphologically and cytologically suggest a possible hybrid origin. The Brithdir specimen ($2n=66$) is almost certainly a hybrid between *R. japonica* ($2n=88$) and *R. sachalinensis* ($2n=44$) and the plants at Preston and Pont Ryhd Sarn may well be of the same origin. Three tetraploid plants were also found and, although superficially similar to *R. japonica*, there are signs that these too may be interspecific hybrids. The clone at South Wylam ($2n=44$) may be of hybrid origin at the tetraploid level. Work is now in progress in comparing these plants morphologically and cytologically with plants produced by controlled pollinations between the two species, and between ploidy levels within *R. japonica*.

All voucher specimens are in LTR.

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REFERENCES

- CONOLLY, A. P. (1977). The distribution and history in the British Isles of some alien species of *Polygonum* and *Reynoutria*. *Watsonia*, 11: 291-311.
 FEDOROV, A. A. (1969). *Chromosome numbers of flowering plants*. Leningrad.
 MOORE, R. J., ed. (1973). *Index to plant chromosome numbers, 1967-1971*. Utrecht.
 MOORE, R. J., ed. (1977). *Index to plant chromosome numbers for 1973-74*. Utrecht.

J. P. BAILEY & A. P. CONOLLY
 Botany Department, The University, Leicester, LE1 7RH

Short Notes

PUTATIVE *REYNOUTRIA JAPONICA* HOUTT. × *FALLOPIA BALDSCHUANICA* (REGEL) HOLUB HYBRIDS DISCOVERED IN BRITAIN

Reports that seed taken from naturalized plants of *Reynoutria japonica* Houtt. in Britain is frequently the result of hybridization with the commonly grown garden plant *Fallopia baldschuanica* (Regel) Holub (Russian Vine) have been the subject of two B.S.B.I. exhibition meeting displays (Bailey & Conolly 1984; Bailey 1987). These exhibits were accompanied by pleas to those interested to try to find such plants growing in the wild. Experience at Leicester had shown that although hybrid seed was capable of surviving the winter and germinating in the spring, such seedlings would be unlikely to survive a British winter, and so the best chance would be to look for seedlings under *R. japonica* plants in spring. These requests have, I am happy to report, borne fruit, and have resulted in B.S.B.I. member D. Bevan, who had seen our exhibits, finding the first *R. japonica* × *F. baldschuanica* growing in the wild; furthermore, the discovery was not just of a seedling, but of a clump of some considerable size, at Railway Fields, Haringey, Middlesex, v.c. 21 (GR 51/317.882) in 1987. This has prompted me to give a fuller account of the discovery, incidence and morphology of this new addition to the British flora.

Bailey & Conolly (1985) reported that the most commonly found variant of *R. japonica* in Britain was octoploid ($2n=88$) and female. Persistent reports that such plants were capable of setting some seed in the absence of male-fertile *Reynoutria* plants led to comparative examination of the chromosome complements of the octoploids with their seedling offspring from eleven localities in Britain (Table 1).

TABLE 1. LOCALITIES OF OCTOPLOID FEMALE *R. JAPONICA* PLANTS FROM WHICH SEED WAS COLLECTED AND GROWN, AND THE NO. OF SUCH SEEDLINGS THAT HAD 54 CHROMOSOMES

Location			No seedlings with $2n = 54$	No seedlings grown
V.c. 11, S. Hants.	Petersfield	GR 41/744.234	5	5
V.c. 12, N. Hants.	Itchen Abbas	GR 41/541.329	5	5
V.c. 40, Salop	Ironbridge	GR 33/671.033	3	4
V.c. 48, Merioneth	Boston Lodge	GR 23/589.382	4	5
V.c. 48, Merioneth	Tyn Coed	GR 23/67.18	2	2
V.c. 48, Merioneth	Ynys	GR 23/597.353	2	2
V.c. 49, Caerns.	Criccieth	GR 23/492.381	4	4
V.c. 49, Caerns.	Pentre'r-felin	GR 23/526.396	3	3
V.c. 49, Caerns.	Pwllheli	GR 23/374.350	5	6
V.c. 55, Leics.	Sileby	GR 43/602.153	4	4
V.c. 55, Leics.	Stoughton	GR 43/644.026	3	3 ^a

^a artificial hybridization with *F. baldschuanica*

Since the female parent, *R. japonica*, had in all the above localities 88 chromosomes, it was something of a surprise that all seedlings counted had 54 chromosomes. Furthermore, whatever was going on was a very regular and widespread phenomenon, occurring as it did in widely separated parts of the country. Cytologically the seedling karyotype was rather distinctive in that ten large chromosomes could be readily distinguished from 44 smaller and more uniform chromosomes typical of *Reynoutria*. Working on the assumption that these seeds were the result of a fertilization event (rather than some bizarre meiotic aberration) one would be looking for a pollen parent with 20 chromosomes. The cytological data in combination with the leaf shape and the semi-twining habit of the seedlings pointed to the involvement of the diploid climber *F. baldschuanica* ($2n=20$) as a putative pollen donor.

Artificial hybridization between a female *R. japonica* from Stoughton and *F. baldschuanica* gave rise to several seeds; three seedlings were subsequently grown on and found to have the same chromosome number and karyotype as well as being virtually identical morphologically with the plants grown from wild-collected seed. A further indication of the ubiquity of this phenomenon came later, when I contacted Richard Scott of I.T.E., Merlewood, who had been conducting research into the suitability of *Reynoutria* taxa as biomass producers. During the course of this work he had collected seeds from female *R. japonica* growing next to a plant of *R. sachalinensis*, thinking not unnaturally that they would grow into the interspecific hybrid. However, when I examined these plants it quickly became apparent that they were hybrids between *R. japonica* and *F. baldschuanica*.

Plants grown outdoors at Leicester for three years are becoming more vigorous but are extremely reluctant to flower; some flower-buds were initiated on one plant this year (1987) but were aborted before they reached maturity. In contrast, the plant at Haringey is extremely vigorous, covering over 10 m², and is strongly rhizomatous and with several densely flowered inflorescences. Judging from its size, it must have been established for some time, since it is many times larger than the three-year-old Leicester plants.

DESCRIPTION OF *R. JAPONICA* × *F. BALDSCHUANICA*

Superficially similar to *R. japonica*, but with stems much thinner and with smaller leaves. Stems herbaceous, up to 2 m long, slender with red blotches, bending over almost to touch the ground, reducing the height of the plant to c. 1.5 m. Leaves acuminate, ovate to narrowly ovate-oblong, to 13 × 6.5 cm; petioles slender, 2–2.5 cm long. Inflorescence of axillary and terminal panicles. Flowers hermaphrodite (?), resembling *R. japonica* more than *F. baldschuanica*; the three outer perianth segments more broadly keeled than in *Reynoutria*; anthers 0.6–0.7 mm long; style trifid, with fimbriate, club-shaped stigmas (intermediate between the fimbriate and capitate stigmas of its respective parents). Younger plants without rhizomes, or only weakly rhizomatous; established clumps may have tough, woody rhizomes up to 2 cm in diameter. Flowering occurs very late in the season (late September to early October); there are no reports of any seed set in this hybrid.

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REFERENCES

- BAILEY, J. P. & CONOLLY, A. P. (1984). A putative *Reynoutria* × *Fallopia* hybrid from Wales. *Watsonia*, 15: 162–163.
 BAILEY, J. P. & CONOLLY, A. P. (1985). Chromosome numbers of some alien *Reynoutria* species in the British Isles. *Watsonia*, 15: 270–271.
 BAILEY, J. P. (1987). *Reynoutria* hybrids in the British Isles. *B.S.B.I. News*, 45: 36.

J. P. BAILEY
 Department of Botany, The University, Leicester, LE1 7RH

References.

- Abraham, Z. & Prasad, P. (1983). A system of chromosome classification and nomenclature. Cytologia 48: 95-101.
- ✓ Ainsworth, C.C., Parker, J.S. & Horton, D.M. (1983). Chromosome variation and evolution in Scilla autumnalis. Kew Chr. Conf. II. George Allen and Unwin. pp 261-268.
- ✓ Bailey, J.P. & Conolly, A. (1985). Chromosome numbers of some alien Reynoutria species in the British Isles. Watsonia 15: 269-277.
- Bailey, J.P. (1988). Putative R. japonica Houtt. x Fallopia baldschuanica (Regel) Holub hybrids discovered in Britain. Watsonia 17: 163-181.
- Bean, W.J. (1976) Trees and shrubs hardy in the British Isles. John Murray, London. Eighth edition, 2nd Impression.
- Bennett, M.D. (1983). The spatial distribution of chromosomes. Kew Chr. Conf. II. George Allen and Unwin. pp71-79.
- ✓ Bennett, M.D. (1984). The genome, the natural karyotype, and biosystematics. Plant Biosystematics. Academic Press, Canada. pp 41-46.
- Bennett, M.D. & Smith, J.B. (1976). Nuclear DNA amounts in angiosperms. Phil. Trans. Roy. Soc. London. B. 274: 227-274.
- Bennett, M.D., Smith, J.B. & Heslop-Harrison, J.S. (1982). Nuclear DNA amounts in angiosperms. Proc. Roy. Soc. London. Ser. B 216: 179-199.
- Bentham, G. & Hooker, J.D. (1880). Genera Plantarum. 3 (1) London.
- Bentzer, B., Bothmer, R., Engstrand, L., Gustafsson, M. & Snogerup, S. (1971). Some sources of error in the determination of arm ratios of chromosomes. Bot. Notiser. 124: 65-74.
- Bolkhovskikh, Z., Grif, V., Matvejeva, T. & Zakharyeva, O. (1969). Chromosome numbers of flowering plants. Leningrad.
- Brandham, P.E. (1983). Evolution in a stable chromosome system. Kew Chr. Conf. II. George Allen and Unwin. pp 251-260.
- ✓ Callaghan, T.V., Lawson, G.J. & Scott, R. (1981). Ecology in the 1980's, natural vegetation as a renewable energy resource in Great Britain. I.T.E. Merlewood, Cumbria.
- ✓ Callaghan, T.V., Lawson, G.J. & Scott, R. (1984). Natural vegetation as a renewable energy resource in Great Britain.

- Longer research report I.T.E., Merlewood, Cumbria.
- Caspersson, T., Farber, S., Foley, G.E., Kudynowski, J., Modest, E.J., Simonsson, E., Wagh, U. & Zech, L. (1968). Chemical differentiation along metaphase chromosomes. Exp. Cell. Res. 49: 219-222.
- Charlesworth, B. & Charlesworth, D. (1978). A model for the evolution of dioecy and gynodioecy. American Naturalist 112: 975-997.
- Chrtek, J. & Chrtková, A. (1983). Reynoutria x Bohemica, Novy Krieznec Z celedi Rdesnovitych. J. Nat. Mus. Praha Hist. Nat. 152: 120.
- Chu, M. & Harberd, D.J. (1970). Note on visual distinction of fluorescent callose of pollen tubes and sieve tubes in stylar tissue of Brassica and its allies. Euphytica 19: 379-381.
- ✓ Conolly, A.P. (1977). The distribution and history in the British Isles of some species of Polygonum and Reynoutria. Watsonia 11: 291-311.
- ✓ Cronquist, A. (1981). An integrated system of classification of flowering plants. Columbia University Press, New York.
- (Dahlgren, R. (1977). A commentary on a diagrammatic presentation of the Angiosperms in relation to the distribution of character states. Pl. Syst. Evol. Suppl. 1: 253-283.
- Dammer, U. (1983). Polygonaceae. In: A. Engler und K. Prantl, Die natürl. Pflanzenfam. 3 (1a): 1-36. Leipzig.
- Danser, B.H. (1926). Die systematische stellung um Reynoutria und Truellum. Bull. Jard. Bot. Buitenzorg, Ser III. T. VIII: I, Buitenzorg.
- Darlington, C.D. (1965). Cytology. Churchill, Ltd. London.
- Darlington, C.D. & LaCour, L.F. (1940). Nucleic acid starvation of chromosomes in Trillium. J. Genet. (London). 40: 185-213.
- Darwin, C. (1884). The different forms of flowers on plants of the same species. 3rd. edition. Murray, London.
- Deumling, B. & Greilhuber, J. (1982). Characterisation of heterochromatin in different species of the Scilla siberica group (Liliaceae) by in situ hybridization of satellite DNA's and flurochrome banding. Chromosoma (Berl.) 84: 535-555.
- DeVriese, W.H. (1849). Polygonum cuspidatum. Jaarb. Koninkl. Nederl. Maats. Aanmoed. Tunib. pp 30-32 & plate.
- De Wet, J.M.J. (1971). Reversible tetraploidy as an evolutionary mechanism. Evolution 25: 545-548.

- Doida, Y. (1960). Cytological studies in Polygonum and related genera. Bot. Mag. (Tokyo) 37: 337-340.
- Dewry, A. (1982). G-banded chromosomes in Pinus resinosa. J. Heredity 73: 305-306.
- Dumortier, B.C. (1827). Florula Belgica. Tornaci Nerviorum.
- Freeman, D.C., Harper, K.T. & Charnov, E.L. (1980). Sex changes in plants: old and new observations and new hypotheses. Oecologia (Berl.) 47: 222-232.
- Fukuda, I. (1984). Chromosome banding and biosystematics. Plant Biosystematics. Academic Press, Canada.
- Gale, E.F., Cundliffe, E., Reynolds, P.E., Richmond, M.H. & Waring, M.J. (1981). Inhibitors of nucleic acid synthesis. In: Molecular basis of antibiotic action. (2nd Ed.) John Wiley and Sons Ltd.
- Gleason, H.A. (1963). New Britton and Brown illustrated Flora vol 2. New York Botanic Garden. New York.
- Goldblatt, P. (1981). Index to plant chromosome numbers 1975 - 1978. Missouri.
- Goldblatt, P. (1984). Index to plant chromosome numbers 1979 - 1981. Missouri.
- Goldblatt, P. (1985). Index to plant chromosome numbers 1982 - 1983. Missouri.
- Graham, S.A. & Wood, C.E. Jr. (1965). The genera of Polygonaceae in the southeastern United States. Journal of the Arnold Arboretum. 46: 91- .
- Greene, E. (1904). Leaflet. Bot. Obsn. Crit. 1: 17-50.
- Greilhuber, J. (1977). Why plant chromosomes do not show G-bands. Theor. Appl. Genet. 50: 121-124.
- Greilhuber, J. (1979). C-band distribution, DNA content and base composition in Adoxa moschatellina (Adoxaceae), a plant with cold-sensitive chromosome segments. Pl. Syst. Evol. 131: 243-259.
- Greilhuber, J. (1986). Severely distorted feulgen -DNA amounts in Pinus (Coniferophytina) after nonadditive fixations as a result of meristematic self-tanning with vacuole contents. Can. J. Genet. Cytol. 28: 409-415.
- Grintzesco, J. (1952). In: T. Sadvulescu, Flora Republicii Populare Romane - Buchavesti.
- Gross, H. (1913). Beiträge zur kenntnis der Polygonaceen. Bot. Jahrb. 49: 234-339.

- Gustaffson, A. & Hakansson, A. (1942). Meiosis in some Rosa hybrids. Bot. Not. Lund. 95: 331-343.
- Haga, T. & Kurabayshi, M. (1954). Genome and polyploidy in the genus Trillium V. Chromosomal variation in natural populations of Trillium kamtschaticum. Pall. Mem. Fac. Sci. Kyushu Univ. El.: 159-185.
- Hara, H. (1966). The flora of eastern Himalaya. University of Tokyo Press.
- Haraldson, K. (1978). Anatomy and taxonomy in Polygonaceae subfam. Polygonoideae Meisn. Emend. Jaretzky. Symbolae Botanicae Upsalienses XXII: 2.
- Hedburg, O. (1946). Pollen morphology in the genus Polygonum L.S. Lat. and its taxonomical significance. Svensk Botanisk Tidskrift. Bd 40 H. 4: 371-404.
- Hooker, J.D. (1880a). Polygonum compactum. Bot. Mag. 106: t. 6476.
- Hooker, J.D. (1880). Polygonum cuspidatum. Bot. Mag. 106: t. 6503.
- Hooker, J.D. (1881). Polygonum sachalinense. Bot. Mag. 107: t. 6540.
- Hooker, J.D. (1897). Polygonum baldschuanicum. Bot. Mag. 123: t. 7544.
- ✓ Holub, J. (1971). Fallopia Adans. 1763 instead of Bilderykia Dum. 1827. Folia Geobot. Phytotax., 6: 171-177.
- Holub, J. (1974). New names in Phanerogamae 3. Folia Geobot. Phytotax 9: 261-275.
- ✓ Houttuyn, F. (1777). Natuurlyke Historie 8. De Kruiden, Amsterdam.
- Hultén, E. (1971). The Circumpolar plants II Dicotyledons. Almquist and Wiksell, Stockholm.
- Hutchinson, J., Flavell, R.B. & Jones, J. (1981). Physical mapping of plant chromosomes by in situ hybridization. In; 'Genetic Engineering' vol. 3 . Plenum Publishing, New York. pp207-222.
- Ishida, M.R. (1961). A cytochemical study of nucleic acids in plant cells. VII. Causal analysis of negative Feulgen staining. Memoirs of the college of Science, Univ. of Kyoto, Series B Vol XXVIII No 1.
- ✓ Jackson, P. & Turtle C. (1986). 'The biology and control of Japanese knotweed Reynoutria japonica, with particular

- reference to Telford. Telford Nature Conservation Project, Stirchley Grange.
- Jackson, R.C. (1982). Polyploidy and diploidy: new perspectives on chromosome pairing and its evolutionary implications. Amer. J. Bot. 69: 1512-1523.
- Jackson, R.C. (1984). Chromosome pairing in species and hybrids. Plant Biosystematics, Academic Press, Canada.
- Janchen, (1960). Cat. Fl. Austr. I. Pteridophyta and Anthophyta. Heft, 4: 912.
- Jamieson, G. Evans, I.J. & Barnes S.R. (1986). An enzymatic method of preparing plant chromosomes for in situ hybridization. Stain Technology 61:21-25.
- Jaretsky, R. (1926). Beitrage zur systematik der Polygonaceae unter Berucksichtigung des oxymethyl-anthrachinon-vorkommens. Feddes Repert. Spec. Nov. Regni Veg. 22: 49-83.
- Jarezky, R. (1928) Histologische und karyologische studien an Polygonaceen. Jahrb. Wissensch. Bot. 69: 357-490.
- Jenkins, G. (1985). Synaptonemal complex formation in hybrids of Lolium temulentum x L. perenne. Chromosoma (Berl.) 92: 81-88.
- John, B. & Lewis, K.R. (1965). Protoplasmatologia VI. Kern-und Zellteilung F. Die chromosomen in der meiose i) The meiotic system. Springer Verlag.
- Johnson, G.D. & Nogueira, D.E.C. (1981). Use of a new antifade buffer for fluorescent staining. J. Immuno. Methods 43: 349-350.
- Jones, K. (1984). Cytology and biosystematics: 1983 pp25-39. In: Plant Biosystematics ed. W.F. Grant.
- ✓ Jones, R.N. & Rees, H. (1968). Nuclear DNA variation in Allium. Heredity 23: 591-605.
- Justice, O.L. (1941). A study of dormancy in seeds of Polygonum. Cornell University Agric. Exp. Station Memoir 235 Icattha, New York.
- Kapoor, B.M. & Gervais, C. (1982). Liste annotée de nombres chromosomiques de la flore vasculaire du nord-est de l'Amerique. 3. Naturaliste Canad. 109: 91-101.
- Kaul, M.L.H. (1988). Male sterility in higher plants. Springer Verlag, Berlin.
- Kay, Q.O.N. & Stevens, D.P. (1986). The frequency distribution and reproductive biology of dioecious sp. in the native flora of Britain and Ireland. Bot. Linn. Soc. 92: 39-64.

- Kimura, Y. & Kitamura, S. (1977). A guide to Flora Japonica of P.F. Siebold. Johnson Reprint Corp.
- Kinosita, K. (1987). Salix sachalinensis forests in the upper Nifungowa river, Kii peninsula. J. Phytogeogr. and Taxon. XXXV; 3: 151-158.
- Komarov, V.L. (ed.) (1936, orig. Russian). Flora of the USSR volume V. Israel program for scientific translations, Jerusalem, 1970.
- Kurita, M & Kuroki, Y. (1970). Y - chromosome and heterochromatin in Rumex acetosa. Jap. J. Genet. 45: 255-260.
- Lai, M.J. (1976). Polygonaceae. In vol II Flora of Taiwan. Ed. Li, H-L. Taipei, Taiwan.
- Laubengayer, R.A. (1937). Studies in the anatomy and morphology of the polygonaceous flower. Am. J. Bot. 24: 329-343.
- Lee, Y.N. (1972). Chromosome number of flowering plants in Korea(4). Journal Korean research Inst. Better Living 8: 41-51.
- Leeman, U. & Ruch, F. (1983). DNA and base content in the nuclei and sex chromatin of Rumex acetosa. Bot. Helv. 93: 77-83.
- Levan, A., Fredga, K. & Sandberg, A.A. (1965). Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220.
- LI, H-L. (1972). Eastern Asia - Eastern North American species - pairs in wide ranging genera. In: Floristics and Paleofloristics of Asia and Eastern North America. Ed. A. Graham 1972. Elsevier Pub. Corp., London, New York. Amsterdam.
- Lima-de-Faria, (1983). Molecular evolution and organization of the chromosome. Elsevier, Amsterdam.
- Linnaeus, C. von, (1753). Species Plantarum, Ed. 1, Holmiae.
- Linnaeus, C. von, (1762). Species Plantarum, Ed. 2, Holmiae.
- Liu, . (1985). In: Peng-Sheng, Index to plant chromosome numbers reported in Chinese Literature. Shanghai.
- Lloyd, D.G. (1982). Selection of combined versus separate sexes in seed plants. Amer. Nat. 120: 571-585.
- ✓ Locandro, R.R. (1978). Japanese bamboo. Weeds Today 9: 21-22.
- Lousley, J.E. & Kent, D.H. (1981). Docks and knotweeds of the British Isles. B.S.B.I., London.
- Löve, A. (1954). Cytotaxonomical evaluation of corresponding taxa. Vegetatio 5-6 pp 212-224.
- Löve, A. & Löve, D. (1956). Chromosomes and taxonomy of Eastern North American Polygonum. Canad. J. Bot. 34: 501-521.

- Löve, A. & Löve, D. In: Bolkhovskikh et al. 1969, Leningrad.
- Löve, A. & Löve, D. In: I.O.P.B. chromosome number reports LXXIV. Taxon 31: 120-126.
- Löve, A. (1957). Sex determination in Rumex. Proceedings of the genetics society of Canada 2: 31-36.
- MacGregor, H. & Varley, J. (1983). Working with Animal Chromosomes. J. Wiley & Sons.
- Majovsky, J., et.al. (1974). Index of chromosome numbers of the Slovakian flora (part 3). Acta. Fac. Rerum. Nat. Univ. Comenianae Bot. 22: 1-20.
- Makino, T. (1901). Polygonum reynoutria (Houtt) Makino. Bot. Mag. Tokyo XV: 84.
- Marks, G.E. (1966). The origin and significance of intraspecific polyploidy: experimental evidence from Solanum chacoense. Evolution 20: 552-557.
- Maruta, E. (1983). Growth and survival of current-year seedlings of Polygonum cuspidatum at the upper distribution limit on Mt. Fuji. Oecologia (Berl.) 60: 316-332.
- Mayr, E. The nature of colonizations in birds. In: The genetics of colonizing species. Eds. Baker, H.G. & Stebbings, G.L. pp29-43 Academic Press, New York & London.
- Meissner, C.F. (1826). Monographiae generis Polygoni prodromus. Geneva.
- Meissner, C.F. (1856). Polygonaceae. In: A. De Candolle, Prodr. Syst. Nat. Regni. Veg. 14, Paris.
- Melchior, H. (1964). A. Engler's syllabus der pflanzenfamilien, 12th ed. Vol. 2 pp 75-79. Berlin
- Menshikova, (1964). In: Chromosome numbers of flowering plants. Bolkhovskikh et. al., Leningrad.
- Merrill, E.D. (1926). An enumeration of Phillipine flowering plants. Bur. Sci. Phillip. 18 Manila Dept. Agr. and Nat. Resources.
- Merrill, E.D. (1938). A critical consideration of Houttuyn's new genera and new species of plants. J. Arnold Arboretum 19:291-375.
- Merrill, E.D. (1938). On Houttuyn's overlooked binomials. Rhodora 40: 290-291.
- Mizianty, M. (1984). Banding patterns in plant chromosomes. II Bibliography (1970-1980) Anthophyta. Acta. Soc. Bot. Pol. 53: 111-136.

- 577
- Mizianty, M. (1985). Banding patterns in plant chromosomes. II. Bibliography (1970-1980) Anthophyta - Supplement 2. Acta. Soc. Bot. Pol. 54: 193-194.
- Moldenke, H.N. (1933). Nomenclatural notes. Bull. Torr. Bot. Cl. LX: 55-59.
- Moldenke, H.N. (1941). Miscellaneous taxonomic notes. Bull. Torr. Bot. Cl. LXVIII: 675-684.
- Moore, D.M. (1982). Flora Europea check-list and chromosome index. C.U.P. Cambridge.
- Moore, R.J. (1973). Index to plant chromosome numbers. 1967-1971. Utrecht.
- Moore, R.J. (1974). Index to plant chromosome numbers. 1972. Utrecht.
- Moore, R.J. (1977). Index to plant chromosome numbers. 1973-1974. Utrecht.
- Murin, A. (1974). In: Index of chromosome numbers of Slovakian flora. part 4. Acta. Fac. Rerum Nat. Univ. Comenianae, Bot. 23:1-23.
- Nakai, T. (1926). A new classification of Linnaen Polygonum. Rigakkai XXIV: 289-301.
- Nakai, T. (1952). A synoptical sketch of the Korean flora. Bull. Nat. Sci. Mus. 31: 32-34.
- Newbury, H.J. & Possingham, J.V. (1977). Factors affecting the extraction of intact ribonucleic acid from plant tissues containing interfering phenolic compounds. Plant Physiol. 60: 543-547.
- Nitsch, J.P. & Nitsch, C. (1969). Haploids from pollen grains. Science 163: 85-87.
- Numata, (ed.) (1974). Flora and vegetation of Japan.
- Ohwi, J. (1965). Flora of Japan. Smithsonian Institute, Washington, D.C.
- Pardue, M.L. & Gall, J.G. (1970). Chromosomal localization of mouse satellite DNA. Science 168: 1356-1358.
- Perdrigeat, M.E.A. (1900). Anatomie comparée des Polygonées et ses rapports avec la morphologie et la classification. Actes. Soc. Linn. Bordeaux 55: 1-91.
- Ping-Sheng, H. (1985). Index to plant chromosome numbers reported in Chinese literature. Shanghai Nat. Hist. Mus.
- Poore, A. (1982). Controlling Japanese Knotweed. Association of

Countryside Rangers Newsletter No 26.

- Price, H.J. (1988). Nuclear DNA content variation within Angiosperm species. Evol. Trends in Plants 2: 53-60.
- Rafinesque, G.S. (1837). Flora telluriana - Philadelphia. Fasc. 1946.
- Richards, A.J. (1973). The origin of Taraxacum agamospecies. Bot. J. Linn. Soc. 66: 189-211.
- Ronse Decraene, L-P. & Akeroyd, J.R. (1988). Generic limits in Polygonum and related genera (Polygonaceae) on the basis of floral characters. Bot. J. Linn. Soc. 98: 321-371.
- Salisbury, E.J. (1909). The extra-floral nectaries of the genus Polygonum. Annals of Botany XXIII: 230-242 & plates XXIII & XVI.
- Salisbury, Sir, E.J. (1964). Weeds and aliens. (New Naturalist 43) pub. Collins.
- Samuelsson, G. (1929). Polygonaceae - Symbolae Sinicae 7.
- Schmitz, J. & Schrank, K.J. (1985). Die drei Reynoutria-sippen (Polygonaceae) des Aachener Stadtwaldes. Gött. Flor. Rundbr. 19: 17-25.
- Schnack, B. & Fernandez, O. (1946). Números cromosómicos de cuatro especies cultivadas. Bol. Soc. Argentina B 1,4: 28-286.
- Schwarzacher, T., Ambros, P. & Schweitzer, D. (1980). Application of Giemsa banding to orchid karyotype analysis. Pl. Syst. Evol. 134: 293-297.
- Schweitzer, D. (1976) DAPI fluorescence of plant chromosomes prestained with actinomycin D. Exp. Cell. Res. 102: 408-413.
- Schweitzer, D. (1980). Fluorescent chromosome banding in plants: applications, mechanisms and implications for chromosome structure. Proc. 4th John Innes Symposium, Norwich 1979. The Plant Genome. (D.R. Davies & R.A. Hopwood, eds.) pp 61-72.
- / Scott, R. & Marrs, R.H. (1984). Impact of japanese knotweed and methods of control. Aspects of Applied Biology 5:
- ✓ Seal, A.G. (1983). DNA variation in Festuca. Heredity 50: 225-236.
- Siebold & Co Extrait du catalogue... de von Siebold & Comp. á Leyde. Jaarb. Nederl. Maatsch. Tuinbouw. pp38-49.
- Siebold, P.F. (1856). Catalogue raisonné. Prix-courant des plantes et graines du Japon cultivées dans l'establishment de von Siebold and Comp. á Leide. Leide and Bohn (Henry and Cohen).

- Shinners, L.H. (1967). Species of Bilderdykia transferred to Reynoutria. Sida. 3: 117-118.
- ✓ Simmonds, N.W. (1976). Evolution of crop plants. Longman, London. (3rd Edition).
- Sinotô, Y. (1929) Chromosome studies in some dioecious plants, with special reference to the allosomes. Cytologia 1: 109-191.
- Small, J.K. (1933). Manual of the Southeastern flora. New York.
- Smith, (1963). In: Chromosome numbers of flowering plants. Bolkhovskikh et. al. 1969, Leningrad.
- Smith, B.W. (1963). The mechanism of sex determination in Rumex hastatulus. Genetics 48: 1265-1288.
- Smith, B.W. (1969). Evolution of sex-determining mechanisms in Rumex. Chromosomes Today vol 2 (Ed. C.D. Darlington & K.R. Lewis). Oliver and Boyd Ltd. Edinburgh.
- Sokolovskaya, (1960). In: Bolkhovskikh, Z. et. al. Leningrad. (1969).
- Stace, C.A. (1965). Cuticular studies as an aid to plant taxonomy. Bull. Brit. Mus. (Nat. Hist.) Bot. 4: 1-78 & plates.
- Stace, C.A. (1975). Hybridization and the Flora of the British Isles. Academic Press, London.
- ✓ Stace, C.A. (1980). Plant Taxonomy and Biosystematics. Arnold, London.
- Stace, C.A. & Ainscough, M.M. (1984). Continuing addition to the gene-pool of the Festuca rubra aggregate (Poaceae: Poaceae). Pl. Syst. Evol. 147: 227-236.
- Stebbins, G.L. (1950). Variation and Evolution in Plants. Columbia University Press.
- Stebbins, G.L. (1971). Chromosomal evolution in higher plants. Edward Arnold, London.
- Stebbins, G.L. (1985). Polyploidy, hybridization, and the invasion of new habitats. Ann. Mo. Bot. Gard. 72:824-832.
- Stevens, D.P. (1988). On the gynodioecious polymorphism in Saxifraga granulata L. (Saxifragaceae). Biol. J. Linn. Soc. 35: 15-28.
- Steward, A.N. (1930). Contributions from the Gray Herbarium of Harvard University. LXXXVIII. The Polygonaceae of Eastern Asia. Pub. Gray Herbarium of Harvard University, Cambridge Massachusetts.
- Sugiura, T. (1931). A list of chromosome numbers in angiospermous plants. Bot. Mag. (Tokyo) 45: 353-355.

- Sugiura, T. (1936). Studies on the chromosome numbers in higher plants, with special reference to cytokinesis, I. Cytologia 7:544-595.
- Suzuka, O. (1950). Chromosome numbers in pharmaceutical plants. I. -Seiken Ziho (Rept. Kihara Inst. Biol. Res.) 4:57-58.
- Takehisa, S. & Utsumi, S. (1973). Heterochromatin and Giemsa banding of metaphase chromosomes in Trillium kamtschaticum Pallas. Nature (New Biol) 243: 286-287.
- ✓ Takhtajan, A. (1980). Outline of the classification of flowering plants. Bot. Rev. 46: 225-359.
- Tanaka, H. (1966). The insect visitors of Polygonum cuspidatum Sieb. et Zucc. Collecting and Breeding 28: 141-143.
- ✓ Taylor, H. (1987). Dyfed Triffids. West Wales Trust for Nature Conservation Bull. 44.
- ✓ Thorne, R.F. (1983). Proposed new realignements in the angiosperms. Nordic J. Bot. 3:85-117.
- ✓ Tutin, T.G., Heywood, V.H., Burgess, N.A., Valentine, D.H., Walters, S.M. & Webb, D.A. (1964). Flora Europea I. Polygonaceae. C.U.P. Cambridge.
- Vana, V. (1972). The localization of heterochromatic segments in the chromosomes of Rumex acetosa L. Preslia 44: 316-326.
- Watkins, A.E. (1932). Hybrid sterility and incompatibility. J. Genet. 25:125-162.
- Wcislo, H. (1977). Chromosome Numbers in the genus Polygonum L.s.l. in Poland. Acta. Biol. Cracov. Ser. (Bot). XX: 153-165.
- Webb, D.A. & Chater, A.O. (1963). Generic limits in the Polygonaceae. Feddes. Rep. Berl. 68: 187-188.
- Wilby, A.S. & Parker, J.S. (1986). Continuous variation in Y chromosome structure of Rumex acetosa. Heredity 57:247-254.
- Zhukova, (1967). Karyology of some plants, cultivated in the Arctic-Alpine Botanic garden. In: Plantarum in zonam polarem transportatio Z Leningrad. Ed. N.A. Aurorin. pp139-149.