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CYTOLOGICAL OBSERVATIONS IN RELATION TO THE TAXONOMY OF THE ORCHIDS OF THE NETHERLANDS

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Abstract

1. The Orchids in the Netherlands have been subjected to a cytological investigation.
2. The division of the genera *Orchis* (L.) Klinge into two new genera: *Orchis* (L.) Vermln. and *Dactylorchis* (Kl.) Vermln. (Vermeulen, 1947), could be confirmed.
3. In *Listera ovata* (L.) R. Br. the diploid chromosome number is 34. Deviating numbers 2n = 35 and 2n = 36 were counted. Because aberations in chromosome number do not cause morphological differences these aberations seem to be unimportant.
4. Out of the material investigated it might be concluded that for the moment it does not seem to be correct to consider *Dactylorchis fuchsii* (Druce) Vermln. as a separate species besides *Dactylorchis maculata* (L.) Vermln. It seems more likely that *D. fuchsii* and *D. maculata* represent two types within a complex-species.

Introduction

For a long time the *Orchidaceae* of the temperate regions have had the attention of cytologists. The Dutch botanist Treub was among the first to mention the chromosomes of the Orchidaceae in his study on the mitosis in *Orchis* and *Epipactis* (1879). Some years later Guignard published his investigations on the meiosis of *Listera ovata* (1884).

Already Strasburger (1882) suggested that the chromosome number is generally the same within a species, though closely related species may have the same number.

Since the end of the nineteenth century many investigators have determined the chromosome number of numerous species of plants. For the *Orchidaceae* of the regions of the northern hemisphere the following investigators are to be mentioned: Afzelius (1922, 1943), Barber (1942), Fuchs and Ziegenspeck (1924), Guignard (1884), Hagerup (1938, 1941, 1944a, b, 1945, 1947), Heusser (1938), Hoffmann (1929, 1930), Löve and Löve (1942, 1944, 1956), Mauer (1938), Modelewski (1918, 1938), Müller (1912), MacMahon (1936), Richardson (1933, 1935), Rosenberg (1905), Sokolovskaja and Strelkova (1940, 1960), Staner (1929), Tuschnjakova (1929), Vermeulen (1938, 1947, 1949), Weijer (1952).

Apart from the number, however, the size and the shape of the chromosomes may be a character of importance to taxonomy. The morphology of the chromosomes in *Listera ovata* in particular has been amply investigated, partly on account of the striking differences in size and shape and also because this species was the first to be subjected to thorough cytological investigations (GUIGNARD (1884), ROSENBERG (1905), MÜLLER (1912), TUSCHNJAKOVA (1929), HOFFMANN (1929, 1930), RICHARDSON (1935), MACMAHON (1936).

A more general and extensive work on the morphology of Orchid chromosomes has been published by HEUSSER (1938). His investigations comprised the basitonic Orchids of Switzerland.

With the exception of those belonging to the *Dactylorchis*-group*) and in a lesser degree those of the *Orchis*-group the Orchids of the Netherlands have not been subjected to any extensive cytological research yet. So it is of importance to have a chromosome study on the Orchids still to be found in the Netherlands, also including those that are now extinct there, but until recently, were a part of the local flora.

MATERIAL AND METHODS

The plants were collected in the field, dug out with a considerable quantity of soil and reared in pots in a non-heated greenhouse or in the open.

In the preliminary stage only squash slides were made. Roottips and flowerbuds were used directly as well as after Carnoy fixation. For staining orceine, aceto-carmine or leuco-basic-fuchsin were used. The results, however, were very poor and therefore microtome sections were also made later on. This technique gave excellent results and was, in this case, certainly preferable to the use of squashes.

For the microtome sectioning roottips and also flowerbuds were fixed in Karpechenko and embedded in paraffine. Microtome sections of 15 micron were stained with cristal-violet or according to Heidenhain's haematoxylin method, or in some cases with leuco-basic-fuchsin according to Feulgen. The best slides were obtained with cristal-violet. However, the degree of this staining depended upon the season in which the material was fixed. When cristal-violet gave no satisfactory results one of the other two mentioned staining methods was used.

Drawings of the metaphase plates of the first—or second division of the pollenmothercell or of the pollendivision and/or of the metaphase plates of roottip cells were made with the aid of an Abbé Camera Lucida.

RESULTS

The results with respect to the chromosome numbers are given in the following table. In the first column: the species; in the second: the determined chromosome number; in the third: the data of literature.

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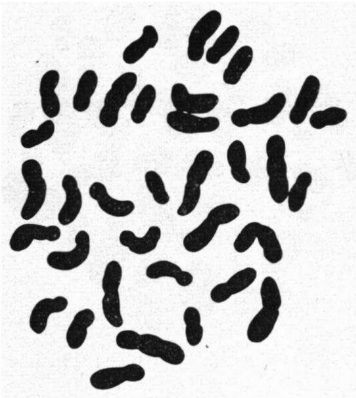
* The name *Dactylorchis* Vermln. (1947) is mostly adopted in modern literature, however, *Dactylorchis* Vermln. is a nomenclatural synonym of *Dactylorhiza* Nevski (1937) (BULLOCK 1959 Taxon 8: 46).

Species	n	2n	Literature	n	2n
Ophrys insectifera L.	18	36, 38	Afzelius 1943	18	—
			Barber 1942	18	—
			Heusser 1938	18	36
Ophrys apifera Huds.	18	36	Barber 1942	18	—
			Heusser 1938	18	36
Orchis morio L.	—	36, 38	Heusser 1938	18	36
			Hagerup 1938	18	—
			Skalinska *et al.* 1957	18	—
			Vermeulen 1947, 1949	18	—
Orchis purpurea Huds.	21	42	Hagerup 1938	21	—
			Heusser 1938	21	42
			Vermeulen 1949	—	42
Orchis simia Lam.	—	42	Heusser 1938	21	42
			Vermeulen 1949	21	—
Orchis militaris L.	21	42	Hagerup 1938	21	—
			Heusser 1938	21	42
			Skalinska *et al.* 1957	—	42
			Vermeulen 1947, 1949	21	—
Orchis mascula L.	—	42	Hagerup 1938	21	—
			Heusser 1938	21	42
			Skalinska 1957	—	42
			Vermeulen 1949	21	—
Dactylorchis fuchsii (Druce) Vermln.	20	40	Afzelius 1958	20	—
			Barber 1942	20	—
			Heslop-Harrison 1948	—	40
			Löve and Löve 1944, 1956	—	40
			Vermeulen 1938, 1947	—	20, 40
Dactylorchis maculata (L.) Vermln.	40	80, 100 120	Barber 1942	40	—
			Hagerup 1938, 1944	—	80
			Heslop-Harrison 1948, 1951	—	80
			Heusser 1938	40	80
			Holman & Kaad 1956	—	80
			Löve 1951	—	80
			Löve and Löve 1956	—	80
			Vermeulen 1938	—	60
			Vermeulen 1947	40	80

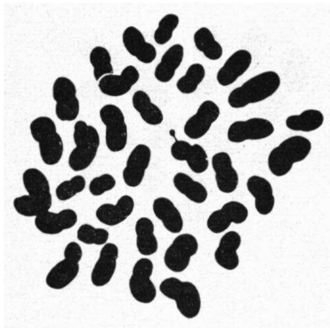
Species	n	2n	Literature	n	2n
Dactylorchis incarnata (L.) Vermln.	20	40	Hagerup 1938	20	—
			Hagerup 1947	—	40
			Heusser 1938	—	40
			Holmen & Kaad 1956	—	40
			Vermeulen 1947	40	80
Dactylorchis praetermissa (Druce) Vermln.	40	80	Maude 1939	—	80, 82
			Vermeulen 1938, 1947, 1949	—	80
Dactylorchis praetermissa (Druce) Vermln. var. junialis (Vermln.) Vermln.	40	80	Vermeulen 1938, 1947	—	80
Dactylorchis majalis (Rchb.) Vermln.	40	80	Hagerup 1938	40	—
			Heusser 1938	—	80
			Skalinska *et al.* 1957	—	80
			Vermeulen 1938	—	80
			Vermeulen 1947	40	80
Gymnadenia conopsea (L.) R. Br.	20	40	Afzelius 1943	20	—
			Barber 1942	20	—
			Heusser 1938	40	40, 80
			Richardson 1935	20	40
			Skalinska *et al.* 1957	—	40
			Sokolovskaja & Strelkova 1960	—	40
Coeloglossum viride (L.) Hartm.	—	40	Afzelius 1943	20	—
			Heusser 1938	—	40
			Löve and Löve 1944, 1956	—	40
			Richardson 1935	20	40
			Sokolovska & Strelkova 1960	—	40
			Skalinska *et al.* 1957	—	40
Platanthera bifolia (L.) Rich.	21	42	Afzelius 1922	21	—
			Heusser 1938	21	42
			Richardson 1935	21	42
			Skalinska *et al.* 1957	—	42
Plathanthera chlorantha (Cust.) Rich.	21	42	Afzelius 1922	21	—
			Hagerup 1947	21	—
			Heusser 1938	—	42
			Richardson 1935	21	42
Anacamptis pyramidalis (L.) Rich.	—	36	Barber 1942	18	—
			Heusser 1938	18	36
			Richardson 1935	21	42
Himantoglossum hircinum (L.) Spreng.	18	—	Heusser 1938	18	36

Species	n	2n	Literature	n	2n
Aceras anthropophorum (L.) R. Br.	21	42	Barber 1942	21	—
			Heusser 1938	21	42
Cephalanthera damasonium (Mill.) Druce	18	—	Barber 1942	18	—
			Hagerup 1947	16	32
			Skalinska *et al.* 1957	16	—
Epipactis palustris (Mill.) Crantz.	20	40	Hagerup 1944	20	—
			Löve and Löve 1944	—	40
Epipactis helleborine (L.) Crantz.	19	38	Barber 1942	19	—
			Hagerup 1945	—	40
			Hagerup 1947	19, 20	—
			Skalinska *et al.* 1959	—	38
			Skalinska *et al.* 1960	—	40
			Weyer 1952	(10) 20	—
Epipactis atrorubens (Hoffm.) Schult.	—	40	Hagerup 1944, 1947	20	—
			Löve and Löve 1944	—	40
Listera ovata (L.) R. Br.	17	34, 35, 36	Barber 1942	17	—
			Guignard 1884	16	—
			Hagerup 1947	17, 18	—
			Hoffmann 1929, 1930	17	—
			Löve and Löve 1944	19	34, 36
			Löve and Löve 1956	—	34, 40
			MacMahon 1936	—	34, 35, 36, 37, 38
			Müller 1912	—	32, 34
			Richardson 1933	—	34, 35, 36
			Rosenberg 1905	16	—
			Skalinska *et al.* 1957	—	34
			Staner 1929	17	—
			Tuschnjakova 1929	16, 17, 18	34, 36
Listera cordata (L.) R. Br.	—	40	Löve and Löve 1956	—	36, 38
			Sokolovskaja & Strelkova 1948	—	38
Neottia nidus-avis (L.) Rich.	18	—	Barber 1942	18	—
			Skalinska *et al.* 1957	—	36
Goodyera repens (L.) R. Br.	15	30	Löve 1954	—	30
			Richardson 1935	15	30
Liparis loeselii (L.) Rich.	—	26	Hagerup 1941	16	—
Hermium monorchis (L.) R. Br.	20	40	Heusser 1938	20	40
Hammarbya paludosa (L.) O.K.	14	—	Hagerup 1944	14	—

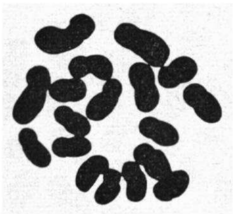


Ophrys insectifera L.
2n = 36

Fig. 1a.

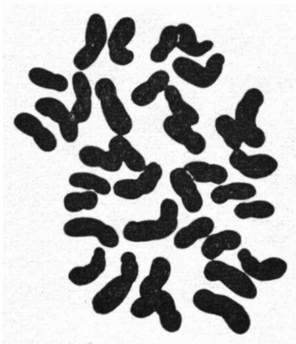


Ophrys insectifera L.
2n = 38

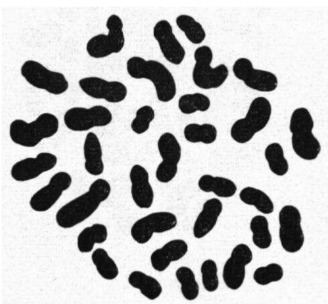


Ophrys apifera Huds.
n = 18

Fig. 1b.

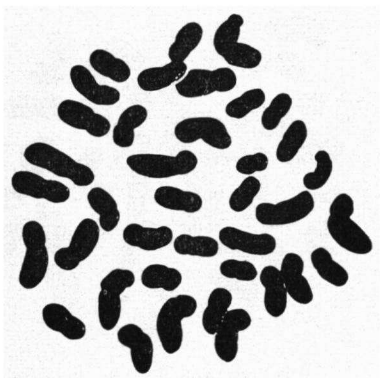


Ophrys apifera Huds.
2n = 36



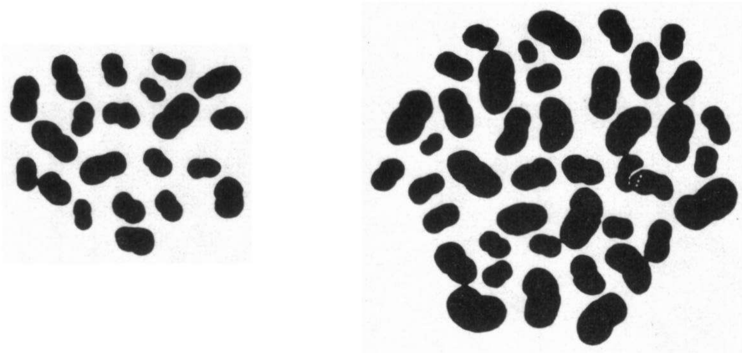
Orchis morio L.
2n = 36

Fig. 1c.



Orchis morio L.
2n = 38

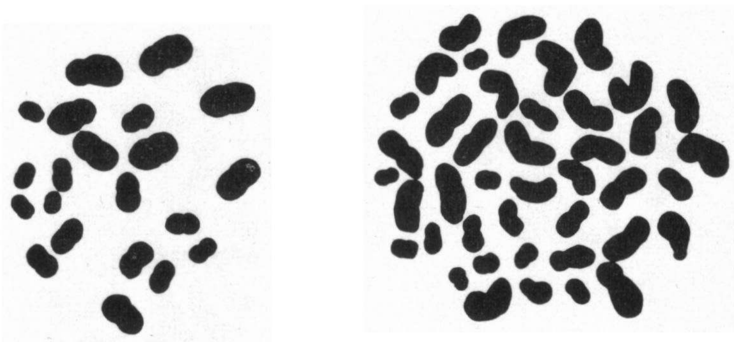
10 μ



Orchis purpurea Huds
n = 21

Orchis purpurea Huds.
2n = 42

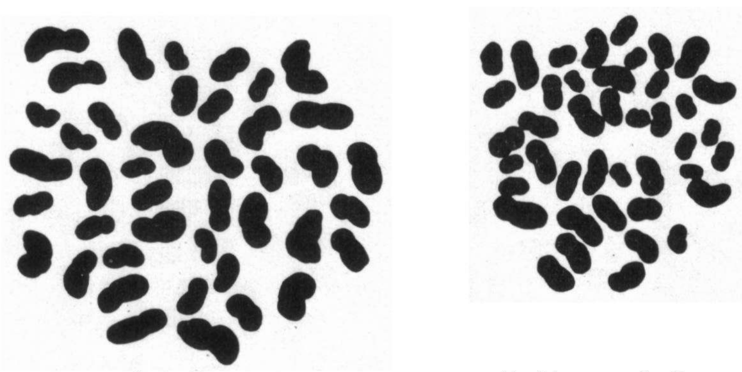
Fig. 2*a*.



Orchis militaris L.
n = 21

Orchis militaris L.
2n = 42

Fig. 2*b*.

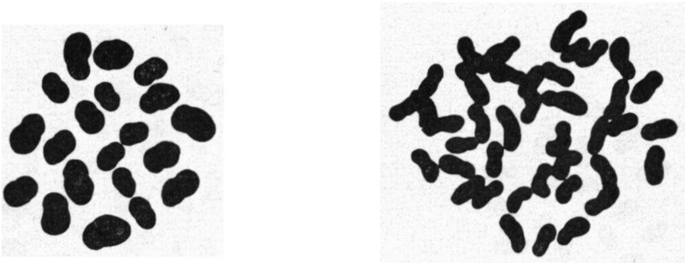


Orchis simia Lamk.
2n = 42

Orchis mascula L.
2n = 42

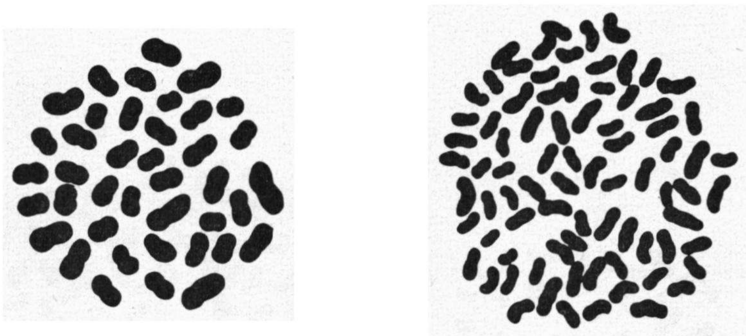
Fig. 2*c*.

10 μ



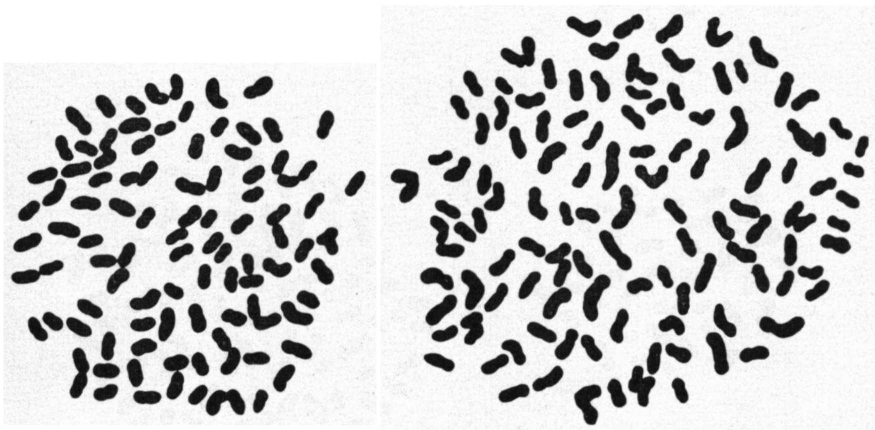
Dactylorchis fuchsii (Druce) Vermln. n = 20 — Dactylorchis fuchsii (Druce) Vermln. 2n = 40

Fig. 3*a*.



Dactylorchis maculata (L.) Vermln. n = 40 — Dactylorchis maculata (L.) Vermln. 2n = 80

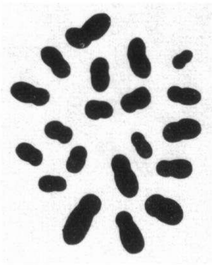
Fig. 3*b*.



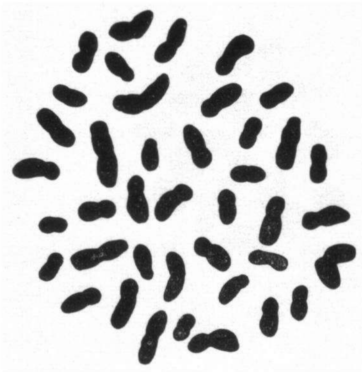
Dactylorchis maculata (L.) Vermln. 2n = 100 — Dactylorchis maculata (L.) Vermln. 2n = 120

Fig. 3*c*.

10 μ

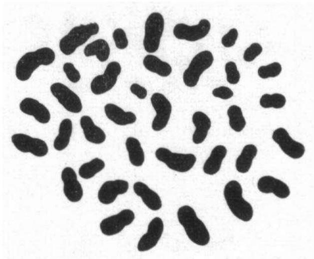


Dactylorchis incarnata (L.) Vermln.
n = 20

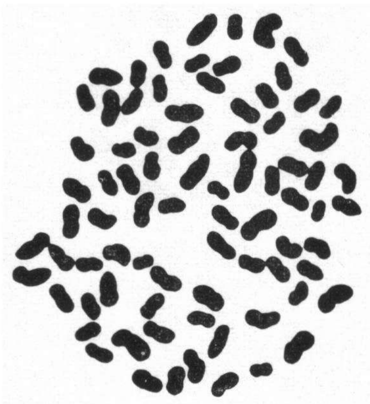


Dactylorchis incarnata (L.) Vermln.
2n = 40

Fig. 4*a*.

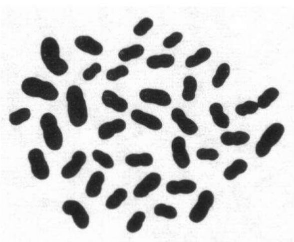


Dactylorchis praetermissa
(Druce) Vermln. n = 40

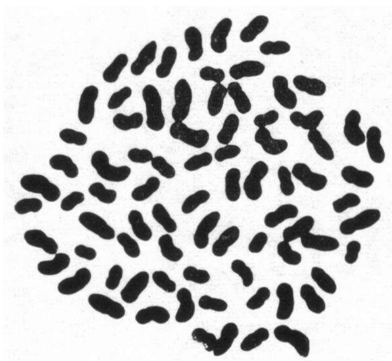


Dactylorchis praetermissa
(Druce) Vermln. 2n = 80

Fig. 4*b*.



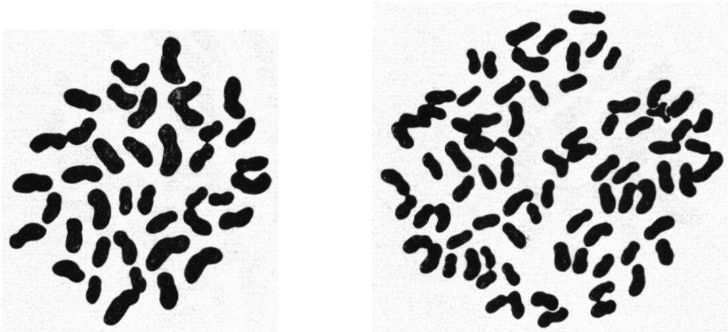
Dactylorchis praetermissa (Druce)
Vermln. var. junialis (Vermln.) Vermln.
n = 40



Dactylorchis praetermissa (Druce)
Vermln. var. junialis (Vermln.) Vermln.
2n = 80

Fig. 4*c*.

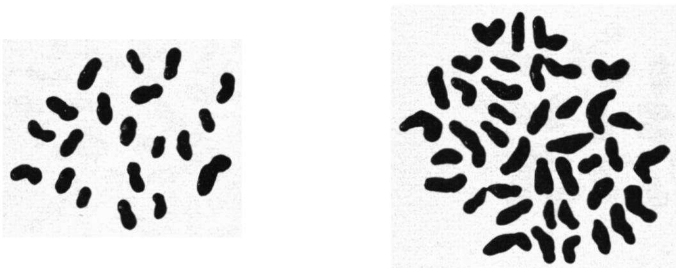
10 μ



Dactylorchis majalis (Rchb.) Vermln.
n = 40

Dactylorchis majalis (Rchb.) Vermln.
2n = 80

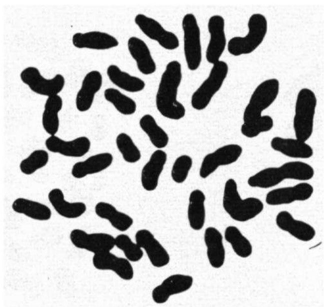
Fig. 5*a*.



Gymnadenia conopsea (L.) R.Br.
n = 20

Gymnadenia conopsea (L.) R.Br.
2n = 40

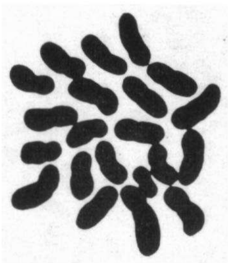
Fig. 5*b*.



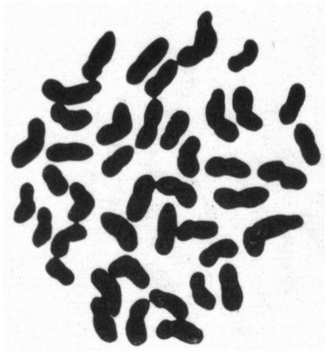
Coeloglossum viride (L.) Hartm.
2n = 40

Fig. 5*c*.

10 μ

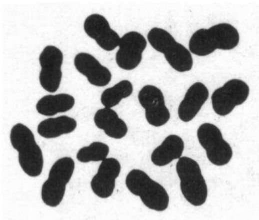


Platanthera bifolia (L.) Rich.
n = 21

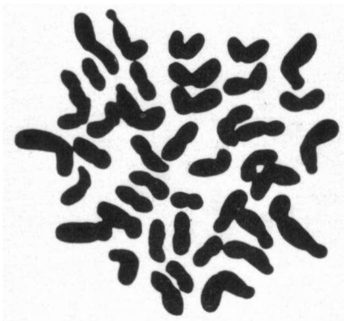


Platanthera bifolia (L.) Rich.
2n = 42

Fig. 6*a*.

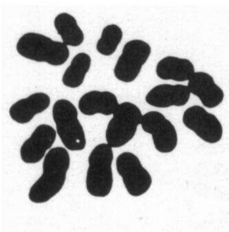


Platanthera chlorantha (Cust.) Rchb.
n = 21



Platanthera chlorantha (Cust.) Rchb.
2n = 42

Fig. 6*b*.

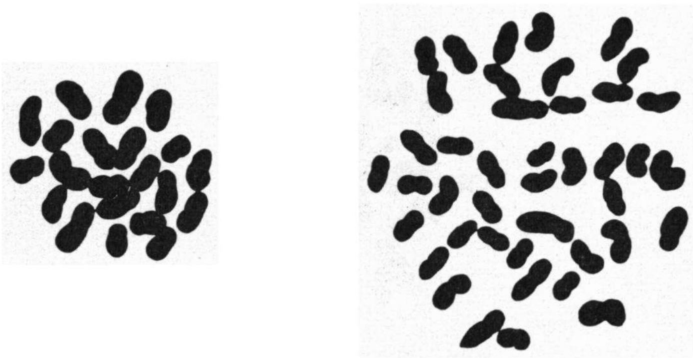


Himantoglossum hircinum (L.) Spreng.
n = 18

Anacamptis pyramidalis (L.) Rich.
2n = 36

Fig. 6*c*.

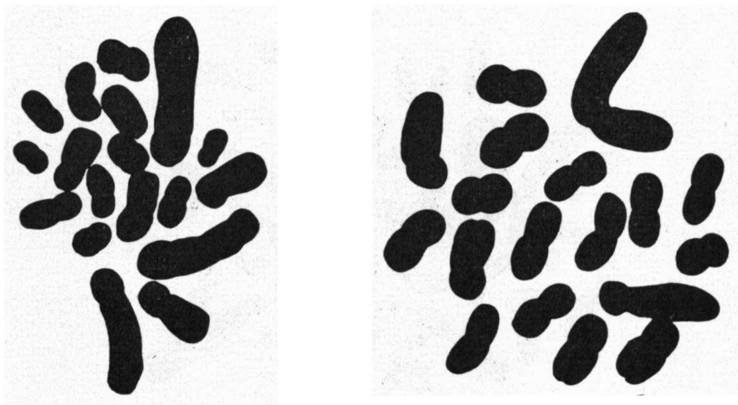
10 μ



Aceras anthropophorum (L.) R.Br.
n = 21

Aceras anthropophorum (L.) R.Br.
2n = 42

Fig. 7*a*.



Cephalanthera damasonium (Mill.) Druce
n = 18

Neottia nidus-avis (L.) Rich.
n = 18

Fig. 7*b*.

10 μ

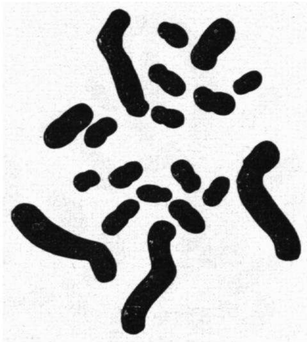
Epipactis palustris (Mill.) Crantz
n = 20

Fig. 8*a*.

Epipactis palustris (Mill.) Crantz
2n = 40

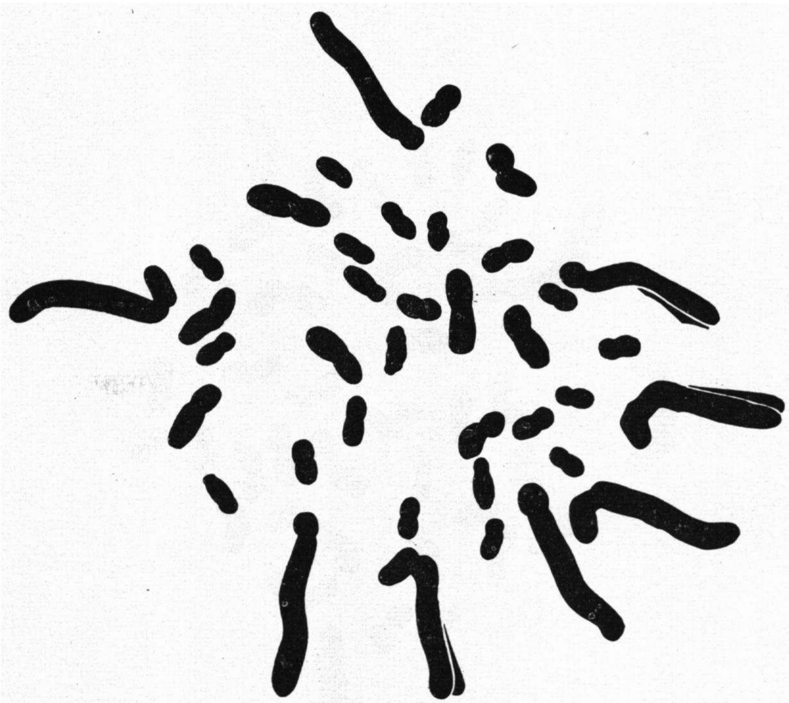
Fig. 8*b*.

10 μ



Epipactis helleborine (L.) Crantz
n = 19

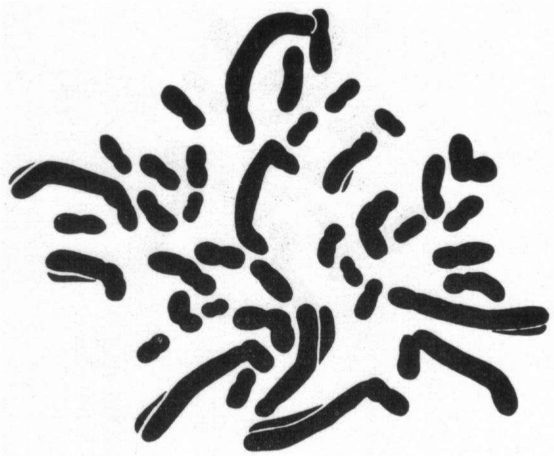
Fig. 9*a*.



Epipactis helleborine (L.) Crantz
2n = 38

Fig. 9*b*.

10 μ



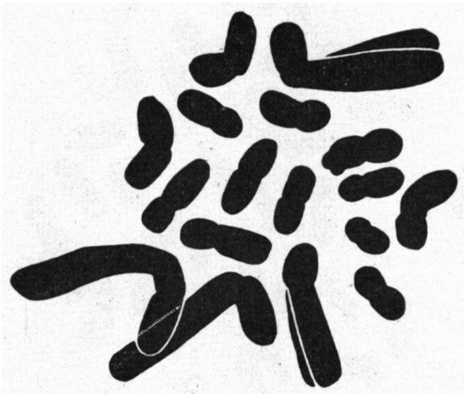
Epipactis atrorubens (Hoffm.) Schult.
2n = 40

Fig. 10*a*.

Listera cordata (L.) R.Br.
2n = 40

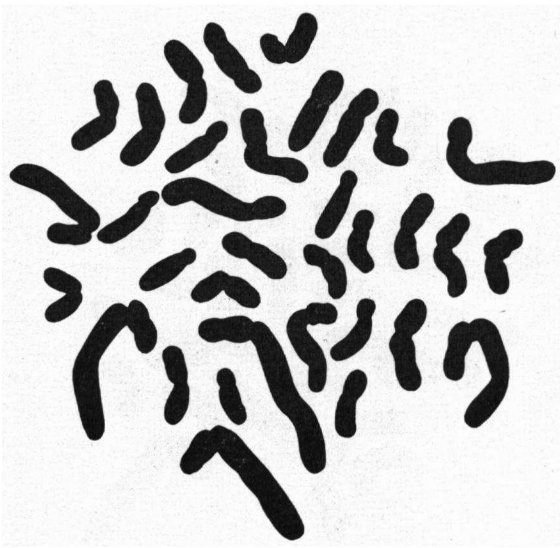
Fig. 10*b*.

10 μ



Listera ovata (L.) R.Br.
n = 17

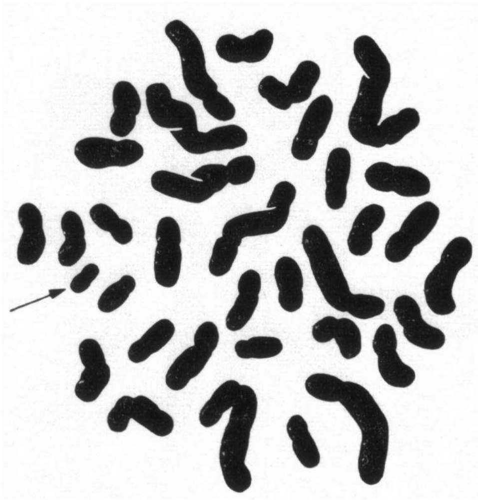
Fig. 11*a*.



Listera ovata (L.) R.Br.
2n = 34

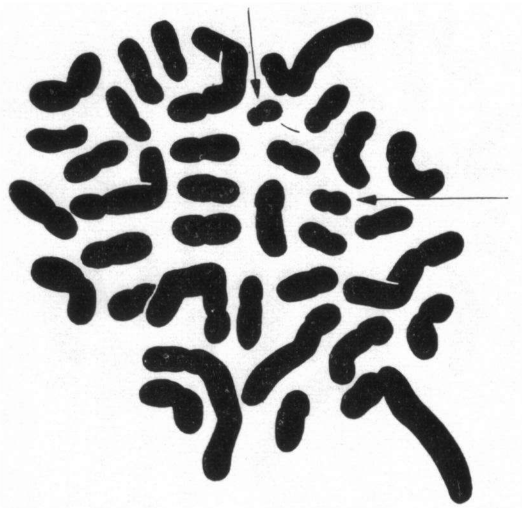
Fig. 11*b*.

10 μ



Listera ovata (L.) R.Br.
2n = 35

Fig. 12*a*.

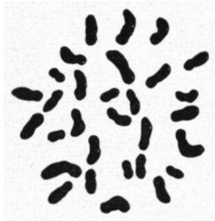


Listera ovata (L.) R.Br.
2n = 36

Fig. 12*b*.

10 μ

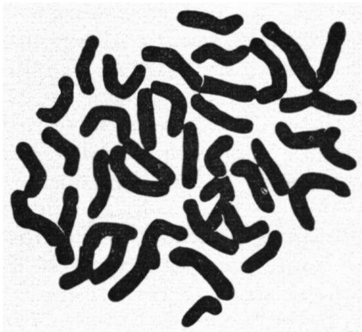
Goodyera repens (L.) R.Br.
n = 15



Goodyera repens (L.) R.Br.
2n = 30

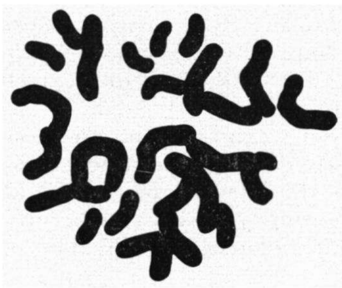
Fig. 13*a*.

Herminium monorchis (L.) R.Br.
n = 20



Herminium monorchis (L.) R.Br.
2n = 40

Fig. 13*b*.



Liparis loeselii (L.) Rich.
2n = 26

Fig. 13*c*.

10 μ

CONCLUSIONS AND DISCUSSION

The family of the *Orchidaceae* is divided into two sub-families:
I. CYPRIPEDIOIDEAE (= DIANDRAE), with two fertile anthers.
II. ORCHIOIDEAE (MONANDRAE), with one fertile anther.

All the *Orchidaceae* found in the Netherlands belong to the second sub-family and this is divided into four tribes (VERMEULEN, 1958).
1. OPHRYDEAE.
2. NEOTTIEAE.
3. EPIDENDREAE
4. VANDEAE. (Not present in the Netherlands).

Of the tribe *Ophrydeae* only one sub-tribe: the *Platantherinae* is present in the Netherlands with the following genera: *Ophrys*, *Orchis*, *Dactylorchis*, *Gymnadenia*, *Coeloglossum*, *Platanthera*, *Anacamptis*, *Himantoglossum*, *Aceras* and *Herminium*.

Of the tribe *Neottia* three sub-tribes: 1. The *Listerinae* with the genera: *Listera* and *Neottia*; 2. the *Cephalantherinae* with: *Epipactis* and *Cephalanthera*; 3. the *Physurinae* with *Goodyera*.

Of the tribe *Epidendreae* only one sub-tribe: the *Liparidinae* with the genera *Hammarbya* and *Liparis*.

With respect to the chromosome size and shape one type of chromosome portrait can clearly be distinguished: no regular pattern, chromosomes belonging to groups with marked differences in size and shape. Some pairs of extremely long chromosomes with sub-terminal centromere are always present.

Distinctly belonging to this type of chromosome portrait are the genera: *Epipactis*, *Listera*, *Cephalanthera* and *Neottia*. (*Epipactis* Zinn with the investigated species *E. palustris* (Mill.) Crantz, 2n = 40, with six long — and two somewhat shorter chromosomes, *E. helleborine* (L.) Crantz, 2n=38, and *E. atrorubens* (Hoffm.) Schult., 2n=40, each with eight long chromosomes; *Listera* R. Br. with *L. ovata* (L.) Br., 2n = 34, 35, 36, always with six extremely long — and two somewhat shorter chromosomes, *L. cordata* (L.) R. Br., 2n=40, with four long chromosomes; *Cephalanthera* Rich., with *C. damasonium* (Mill.) Druce, n=18, with three long chromosomes; *Neottia* Rich. with *Neottia nidus - avis* (L.) Rich., n=18, with one extremely long — and one somewhat shorter chromosome.)

From the other species examined it can be concluded that, though the chromosomes are different in size, these differences are less pronounced than in the species mentioned above. However, some differences in chromosome portrait can be observed. Most distinctly in the metaphase plates of roottip cells. The following groups might be distinguished:

1. *Orchis*, *Ophrys*; 2. *Anacamptis*, *Aceras*, *Coeloglossum*; 3. *Platanthera*, *Gymnadenia*, *Dactylorchis*; 4. *Goodyera* and 5. *Liparis*, *Herminium*. (*Hammarbya* is not included since no preparations clear enough to be drawn could be obtained.)

Group 1–3 more or less form an unity as opposed to group 5. Within the groups 1–3 the difference in chromosome portrait between the genera *Orchis* and *Dactylorchis* is most striking. The general pattern of the chromosomes in *Dactylorchis* shows similarity to that of *Gymnadenia*. On the other hand there is also a difference in the basic-number of the chromosomes. In *Orchis* x = 21, with exception of *Orchis morio* (x = 18), and in *Dactylorchis* x = 20.

These cytological differences are also mentioned by VERMEULEN (1947). Together with the following morphological characters these cytological ones were the arguments in favour of the division of the genus *Orchis* (L.) Klinge into new genera: *Orchis* (L.) Vermln. and *Dactylorchis* (Kl.) Vermln.:

1. The tuber of *Orchis* is simple, whereas *Dactylorchis* has a divided tuber.
2. The bracts are membranaceous in *Orchis* and green (herbaceous) in *Dactylorchis*.
3. The spike of *Orchis* is enclosed by the upper leaves in a spathe-like way until just before the opening of the flowers; the lower leaves form a rosette. *Dactylorchis* has an inflorescence visible already from the beginning and has no basal rosette. Furthermore, hybrids between *Orchis* and *Dactylorchis* are very rare, whereas hybryds between *Dactylorchis* and *Gymnadenia* are found much more frequently. Apperently *Dactylorchis* stands more closely to *Gymnadenia* than to *Orchis*.

The division of *Orchis* (L.) Kl. into *Orchis* (L.) Vermln. and *Dactylorchis* (Kl.) Vermln. as was done by VERMEULEN (1947) is supported by both morphological and cytological characters, and is therefore well founded and acceptable in all respects.

In *Ophrys insectifera*, *Orchis morio* and *Listera ovata*, different chromosome numbers have been determined.

The plants investigated of *Ophrys insectifera* as well as those of *Orchis morio* were collected in the same habitat, growing close to each other. In both instances only two plants were investigated. The numbers 2n = 38 are probably incidental deviations possibly caused by irregularities in meiosis as mentioned by HAGERUP in this paper on the spontaneous formation of aneuploid embryos in Orchids. (1947).

In *Listera ovata* n = 17 and 2n = 34, 35 and 36 were found. In plants with 2n = 35 there is a small additional chromosome, not present in the 2n = 34 individuals, the 2n = 36 plants having two additional small chromosomes. In both cases these additional chromosomes have a centromere.

In some metaphase plates of cells in roottips of plants having 2n = 34 chromosomes a number of 35 was counted, and in one plant in the same roottip the chromosome numbers 2n = 34, 35, 36, 37, 38 and 42 were found.

TUSCHNJAKOVA (1929) investigated the meiosis in *Listera ovata*. She found n = 16, 17 and 18. She also noted the existence of some cells having 2n = 36 in plants with 2n = 34. In the embryosac she observed the formation of gametes with abnormal chromosome numbers as a result of non-disjunction.

A detailed study of the morphology of the chromosomes of *Listera* *ovata* has been carried out by RICHARDSON (1933). She counted 2n = 34, 35 and 36. She concludes that the excessive chromosomes must have originated from fragmentation of bigger ones during meiosis. This conclusion is based on the following arguments: first, the chromosomes are very small, and secondly, they differ morphologically from the other ones by having no centromere. This, however, is in disagreement with the observations of Tuschnjakova. Our own investigations, too, are in contradiction with her observations. The morphology of the small additional chromosomes shows similarity to that of the other chromosomes and centromeres are present.

MACMAHON (1936) studied the behavior of the chromosomes during the first meiotic division; he came to the conclusion that these chromosomes have to be regarded as supernumeraries, corresponding with the "B" chromosomes of other plant groups.

As to *Listera ovata* no correlation between the chromosome number and the habitat has been found; in each habitat 2n = 34, 35 and 36 appear to be present. The habit of *Listera ovata* is very uniform. No morphological differences could be observed in plants with different chromosome numbers.

The conclusion, therefore, must be that the numbers found are independent of environmental factors and that the basic number is x = 17. Aberations in chromosome number not causing any morphological differences and therefore, not seeming important.

In the *Orchidaceae*, as far as is known, polyploidy is rare. MIDUNO (1940) regards this as a character of the family. Yet an example is presented by *Dactylorchis maculata* 2n = 60 (VERMEULEN 1938), 80, 100 and 120 having been found.

When VERMEULEN (1947) segregated *Dactylorchis* from *Orchis*, *Orchis maculata* was placed into this segregate genus on account of morphological and cytological characters. Moreover, Vermeulen distinguished beside *Dactylorchis maculata* (L.) Vermln., *Dactylorchis fuchsii* (Druce) Vermln., which may be separated as follows:

1. As compared to those of *Dactylorchis maculata*, *Dactylorchis fuchsii* has lower leaves which tend to be rounded or blunt and are widest above the middle.
2. The spike of *Dactylorchis fuchsii* is narrower than that of *Dactylorchis maculata.*
3. The labellum of *Dactylorchis fuchsii* is clearly three-lobed, the middle lobe being at least as wide, or wider, as the lateral lobes. In *Dactylorchis maculata* the midlobe is narrower than the lateral lobes.
4. *Dactylorchis fuchsii* has 2n = 20 and *Dactylorchis maculata* 2n = 40.

The difference in leafshape is usually more or less visible, but it is not always very convincing. The shape of the labellum generally is as stated above, but in the plants examined, not all flowers of the same spike match this discription.

Already after one year of cultivation under the same circumstances, the habit of *Dactylorchis maculata* is becoming more like that of *Dactylorchis fuchsii.* The differences mentioned under 1, 2 and 3 are becoming less distinct, particularly the shape of the inflorescence is becoming more cylindrical as in *Dactylorchis fuchsii.*

An ecological investigation showed that *Dactylorchis fuchsii* occurs on calcareous soil, whereas *Dactylorchis maculata* prefers poor acid soils. This confirms an observation of HESLOP-HARRISON (1948, 1951, 1954b).

The distinction between *Dactylorchis fuchsii* and *Dactylorchis maculata,* therefore, seems unclear in some instances. The main differential characters, after all, appear to be the chromosome number and the ecological preference.

From the material examined it might be concluded that for the moment it still seems better to suggest that in the Netherlands *Dactylorchis fuchsii* and *Dactylorchis maculata* constitute two types within a species having a polyploid series, rather than two separate species.

This would be in contradiction with the extensive and thorough investigations in the *Dactylorchis maculata* complex, in Great-Britain and Sweden by HESLOP-HARRISON (1948, 1951, 1954b). In comparison with the amount of material studied by this author very few plants have been investigated. For a final solution of this problem, that seems to exist in the Netherlands a more extensive study of mass-collections as was done by Heslop-Harrison, ought to be undertaken, in addition to experimental taxonomic research.

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