# A CYTOTAXONOMIC STUDY OF VIOLA IN THE NETHERLANDS

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## Abstract

1. The chromosome numbers of 10 species of the genus *Viola* in the Netherlands were determined.
2. *Viola riviniana* has various chromosome numbers: 2n = 35, 40, 45, 46, 47 (most often 2n = 40).
3. It was not possible to find a correlation between the external morphology and the various chromosome numbers in *V. riviniana*.
4. Despite the variability of *V. riviniana* it proved impossible to divide the Dutch material into subspecies.
5. Some differential characters of *V. riviniana* and *V. reichenbachiana* are described.
6. *V. canina* is not variable in cytological respect in the Netherlands.
7. *V. calaminaria* is not related to *V. lutea* but to the *V. tricolor* complex.


## Introduction

Up to the present numerous investigations into the cytology of the genus *Viola* have been carried out. The chromosome numbers of many species are now known; moreover, as much experimental crossing has been done, a combination of morphological, cytological and genetical data is available.

In some cases, however, only a few plants of a species were examined. Therefore, the available information pertained only to a part of the entire area of the species. In the Netherlands none of the species was cytologically examined.

Clausen (1931, b), studying the cytology of *Viola canina* in Denmark, obtained results quite different from those of Bruun (1932) in Sweden, Fothergill (1944) in England and Schöfer (1954) and Schmidt (1961) in Germany. The Dutch material was not examined.

*Viola riviniana* was studied in England by Valentine (1941, 1949, 1950, 1956, 1958) and in South Germany by Schöfer (1954). In England five different chromosome numbers were found: 2n = 35, 40, 45, 46, and 47 respectively (most often 40), whereas in South Germany only the number 2n = 40 was obtained. The British plants produced abundant seed and apparently had not originated through hybridization. In certain plants from British populations 5, 6 or 7 small "supernumerary" or "B" chromosomes were present. According to Schmidt (1961) this has not yet been found in continental plants. Valentine described extensively the British plants of *Viola riviniana* and *Viola reichenbachiana* and their artificially produced hybrid. Schöfer studied South German plants of putative hybrid origin, but gave only inadequate descriptions. Whereas the hybrids produced artificially in Britain all have the number 2n = 30, two types of hybrids were found to occur in South Germany; one, accounting for 90 % of the total, had 2n = 40, the remaining 10 % had 2n = 30. Another point of difference is that the hybrids from Germany proved to be fertile, whereas the British ones were virtually completely sterile.

According to VALENTINE (1941), the British population of *V. riviniana* consists of two morphologically distinct subspecies, a type of woods (*Viola riviniana* Rchb. subspec. *nemorosa* N.W. et M. emend. Valentine) and a type of open grassland [*V. riviniana* Rchb. subspec. *minor* (Murbeck) Valentine]. There are, however, intermediate types from intermediate habitats.

As neither the cytology nor the morphological variability of *V. riviniana* in the Netherlands had been investigated, it seemed worthwhile to undertake a study of these subjects.

Another interesting problem in the Dutch violets is the chromosome number of *Viola calaminaria* Lej. This had never been satisfactorily established, despite several attempts. It is true that GRIESINGER (1937) was able to determine it in the blue zinc-violets of Blankenrode, Westphalia, but morphologically these plants are not identical with those from the South of the Netherlands.

On various grounds HEIMANS (1936) suggested that our yellow-flowered zinc-violets are morphologically closer to the *Viola tricolor* complex (2n = 26) than to *V. lutea* (2n = 48), with which they are usually classified in floras. This conclusion is, however, subject to dispute, as CLAUSEN (1931, *c*) counted 2n = ca. 48 for *V. lutea* Huds. var. *multicaulis* Koch (= *V. calaminaria* Lej.). HEIMANS (1960) found a higher number, 2n = prob. 52, but the exact number could not be established. One of the objectives of the present study was to obtain an exact count of *V. calaminaria* and to compare the species morphologically with *V. tricolor*. Finally, the chromosome numbers of all Dutch species (exept *V. stagnina*) were determined, in order to obtain a more complete picture of the cytogeography of these species.

## MATERIAL AND METHODS

Living plants were collected in the Netherlands and some adjacent regions. All plants were cultivated during 4 years in the Cantonspark at Baarn, the Botanical Garden of the State University of Utrecht. Only living plants were studied morphologically. Herbarium material of most of the plants was deposited in the Utrecht Herbarium.

The chromosome counts were made from mitoses in root-tips. These were fixed in Karpechenko, embedded in paraffin, sectioned at 15 $\mu$, and stained according to the haematoxylin or the gentian violet-iodine method. The chromosome portraits were drawn with the aid of an Abbé Camera Lucida.

## RESULTS

Table 1 gives a summary of the author's chromosome counts, with indication of the origin of the material, the number of plants, and

TABLE 1

| Species | Plant or herbarium number | Number of plants | Origin | Diploid chromosome number |
|---|---|---|---|---|
| Viola odorata L. | 52 | 3 | Prov. S. Holland: near Rockanje | 20 |
| | 103 | 2 | Prov. N. Brabant: near Oisterwijk | 20 |
| | 33 | 3 | Prov. Limburg: near Epen | 20 |
| | 159, 162 | 6 | Prov. Limburg: near Schin op Geul | 20 |
| Viola hirta L. | 54, 282, 286, 290 | 4 | Prov. Limburg: Eyserbos near Eys | 20 |
| | 9 | 3 | Prov. N. Holland: dunes between Bakkum and Egmond | 20 |
| | 52, 151 | 4 | Prov. S. Holland: dunes near Rockanje | 20 |
| | 156 | 2 | Prov. S. Holland: dunes near Oost-Voorne | 20 |
| Viola riviniana Rchb. | | | A. THE NETHERLANDS | |
| | 173 | 6 | Prov. Friesland: near Oude Mirdum | 40 |
| | 174 | 12 | Prov. Drenthe: near Weerdinge | 40 |
| | 180 | 5 | Prov. Drenthe: near Sleen | 47 |
| | 64 | 2 | Prov. Overijssel: near de Lutte | 40 |
| | 129 | 3 | Prov. Overijssel: near Oldenzaal | 40 |
| | 130, 136 | 6 | Prov. Overijssel: near Denekamp | 40 |
| | 135 | 3 | Prov. Overijssel: between Denekamp and Punthuizen | 40 |
| | 145 | 2 | Prov. Overijssel: near Lattrop | 40 |
| | 131 | 1 | Prov. Overijssel: near Oldenzaal | 46 |
| | 118 | 3 | Prov. Gelderland: near Berg en Dal (Nijmegen) | 40 |
| | 217 | 3 | Prov. Gelderland: Ermelo (Leuvenumse bos) | 40 |
| | 213 | 1 | Prov. Gelderland: Putten (Pinetum Schovenhorst) | 45 |
| | 110, 113 | 4 | Prov. Utrecht: Rhenen (Grebbeberg) | 40 |
| | 12 | 3 | Prov. N. Holland: between Bergen and Bergen aan Zee | 40 |
| | 46 | 4 | Prov. S. Holland: Rockanje, in the dunes | 40 |
| | 152, 153, 207 | 6 | Prov. S. Holland: dunes near Rockanje | 40 |
| | 150, 157 | 5 | Prov. S. Holland: dunes near Oost-Voorne | 40 |
| | 297 | 1 | Prov. N. Brabant: near Chaam | 47 |
| | 50 | 1 | Prov. Limburg: Schweiberger Bos near Mechelen | 35 |
| | 165 | 1 | Prov. Limburg: Geerendal near Schin op Geul | 35 |
| | 198 | 1 | Prov. Limburg: Savelsbos near Rijckholt | 35 |
| | 29 | 1 | Prov. Limburg: near Vijlen | 40 |
| | 41 | 1 | Prov. Limburg: near Epen | 40 |
| | 119 | 4 | Prov. Limburg: near Mook | 40 |
| | 160 | 2 | Prov. Limburg: Geerendal near Schin op Geul | 40 |
| | 193 | 1 | Prov. Limburg: Osebos near Gulpen | 40 |
| | 283 | 1 | Prov. Limburg: Eyserbos near Eys | 40 |

TABLE 1 *(Continued)*

| Species | Plant or herbarium number | Number of plants | Origin | Diploid chromosome number |
|---|---|---|---|---|
| | 317, 354, 373, 374, 377, 378, 379, 381, 382, 384, 385, 387, 390, 391, 397 | 16 | Prov. Limburg: on slopes in the "Schweiberger bos" near Mechelen | 40 |
| | 39 | 3 | Prov. Limburg: Bovenste Bos near Epen | 45 |
| | 17 | 3 | Prov. Limburg: Ravensbos near Valkenburg | 46 |
| | | | B. W. GERMANY | |
| | 235 | 1 | Lower Saxony: Bentheim | 40 |
| | 243 | 1 | Lower Saxony: Bentheim | 47 |
| | | | C. BELGIUM | |
| | 201, 202, 203, 204, 284, 285, 286 | 7 | Prov. Liège: Ruïne Reinardstein near Robertville | 40 |
| | 287, 288, 289 | 3 | Prov. Luxembourg: Between Marche en Vamennes and La Roche en Ardennes | 40 |
| | 101 | 3 | Prov. Luxembourg: near Bérismenille | 40 |
| Viola reichenbachiana Jord. ex Bor. | | | A. THE NETHERLANDS | |
| | 4 | 1 | Prov. Overijssel: Austieberg near Denekamp | 20 |
| | 5, 58, 63 | 4 | Prov. Overijssel: Lomanskamp near Ootmarsum | 20 |
| | 37 | 2 | Prov. Limburg: Onderste Bos near Epen | 20 |
| | 191, 192, 194, 195, 196 | 5 | Prov. Limburg: Osebos near Gulpen | 20 |
| | 197 | 1 | Prov. Limburg: Savelsbos near Rijckholt | 20 |
| | 199, 200 | 2 | Prov. Limburg: Eyserbos near Eys | 20 |
| | 355, 372, 375, 376, 380, 383, 386, 388, 389, 392, 393, 394, 395, 396, 397 | 15 | Prov. Limburg: on slopes in the Schweiberger Bos near Mechelen | 20 |
| | | | B. BELGIUM | |
| | 190 | 3 | Prov. Limburg: near Lanaken | 20 |
| | | | C. W. GERMANY | |
| | 229, 236, 237, 238, 245, 247 | 6 | Lower Saxony: near Bentheim | 20 |
| | 240, 241, 244 | 3 | Lower Saxony: Burg Steinfurt near Bagno | 20 |
| | 243, 246 | 2 | Lower Saxony: 3 km North of Bentheim | 20 |
| Viola rupestris F. Schmidt | 205 | 3 | Prov. N. Holland: Bergen aan Zee (collected by Ir. H. Doing Craft) | 20 |
| var. arenaria (D.C.) W. Beck. | 293 | 1 | Prov. S. Holland: Oost-Voorne (collected by C. Sipkes) | 20 |
| Viola canina L. | 208, 209, 210, 211, 212 | 5 | Prov. Friesland: Isle of Schiermonnikoog | 40 |

TABLE 1 *(Continued)*

| Species | Plant or herbarium number | Number of plants | Origin | Diploid chromosome number |
|---|---|---|---|---|
| | 175 | 2 | Prov. Drenthe: Valtherzand near Emmen | 40 |
| | 249 | 1 | Prov. Drenthe: near Oosterhessele | 40 |
| | 96 | 3 | Prov. Overijssel: near Marienberg | 40 |
| | 148 | 1 | Prov. Overijssel: near de Lutte | 40 |
| | 179 | 3 | Prov. Overijssel: near Gramsbergen | 40 |
| | 1 | 3 | Prov. Utrecht: near Soestdijk | 40 |
| | 84, 85, 87 | 9 | Prov. Utrecht: between Maarn and Maarsbergen | 40 |
| | 6 | 4 | Prov. N. Holland: dunes of Texel (de Mok) | 40 |
| | 8 | 1 | Prov. N. Holland: dunes of Texel (near de Koog) | 40 |
| | 181 | 1 | Prov. N. Holland: dunes of Texel (near den Hoorn) | 40 |
| | 70, 73 | 6 | Prov. N. Holland: dunes near Bergen aan Zee | 40 |
| | 81 | 3 | Prov. N. Holland: near St. Maartenszee | 40 |
| | 214 | 3 | Prov. S. Holland: Goudriaen | 40 |
| | 271, 262, 269, 270 | 4 | Prov. S. Holland: near Oost-Voorne | 40 |
| | 265 | 1 | Prov. S. Holland: near Rockanje | 40 |
| | 108, 109 | 6 | Prov. N. Brabant: near Rosmalen | 40 |
| Viola palustris L. | 132 | 3 | Prov. Overijssel: near Denekamp | 48 |
| | 248 | 1 | Prov. Gelderland: near Ermelo | 48 |
| | 83 | 2 | Prov. Utrecht: near Maarn | 48 |
| | 100 | 3 | Belgium. — Prov. Luxembourg: near Mochamps. | 48 |
| Viola tricolor L. a) subspec. tricolor | 172 | 8 | Prov. Friesland: near Oude Mirdum | 26 |
| | 177 | 1 | Prov. Overijssel: near Marienberg | 26 |
| | 92, 99 | 3 | Prov. Overijssel: near Ommen | 26 |
| | 116 | 10 | Prov. N. Holland: between Hollandse Rading and Hilversum | 26 |
| b) subspec. curtisii (Forst.) Syme | 67, 71 | 2 | Prov. N. Holland: near Bergen aan Zee | 26 |
| | 10 | 2 | Prov. N. Holland: near Egmond a/d Hoef | 26 |
| | 48 | 2 | Prov. S. Holland: near Rockanje | 26 |
| | 43 | 2 | Prov. S. Holland: near Oost-Voorne | 26 |
| | 128 | 9 | Prov. S. Holland: near Ouddorp | 26 |
| Viola arvensis Murr. | 176 | 2 | Prov. Drenthe: near Emmen | 34 |
| | 93 | 3 | Prov. Overijssel: near Ommen | 34 |
| | 133 | 3 | Prov. Overijssel: near Denekamp | 34 |
| | 114 | 3 | Prov. Utrecht: near Utrecht | 34 |
| | 216 | 10 | Prov. N. Holland: near Hilversum | 34 |
| | 102 | 2 | Prov. N. Brabant: near Oisterwijk | 34 |
| | 186 | 2 | Prov. N. Brabant: near Valkenswaard | 34 |
| | 105 | 2 | Prov. N. Brabant: near Rosmalen | 34 |
| Viola calaminaria Lej. | 168 | 6 | Belgium. — Prov. Liège: La Calamine | 52 |

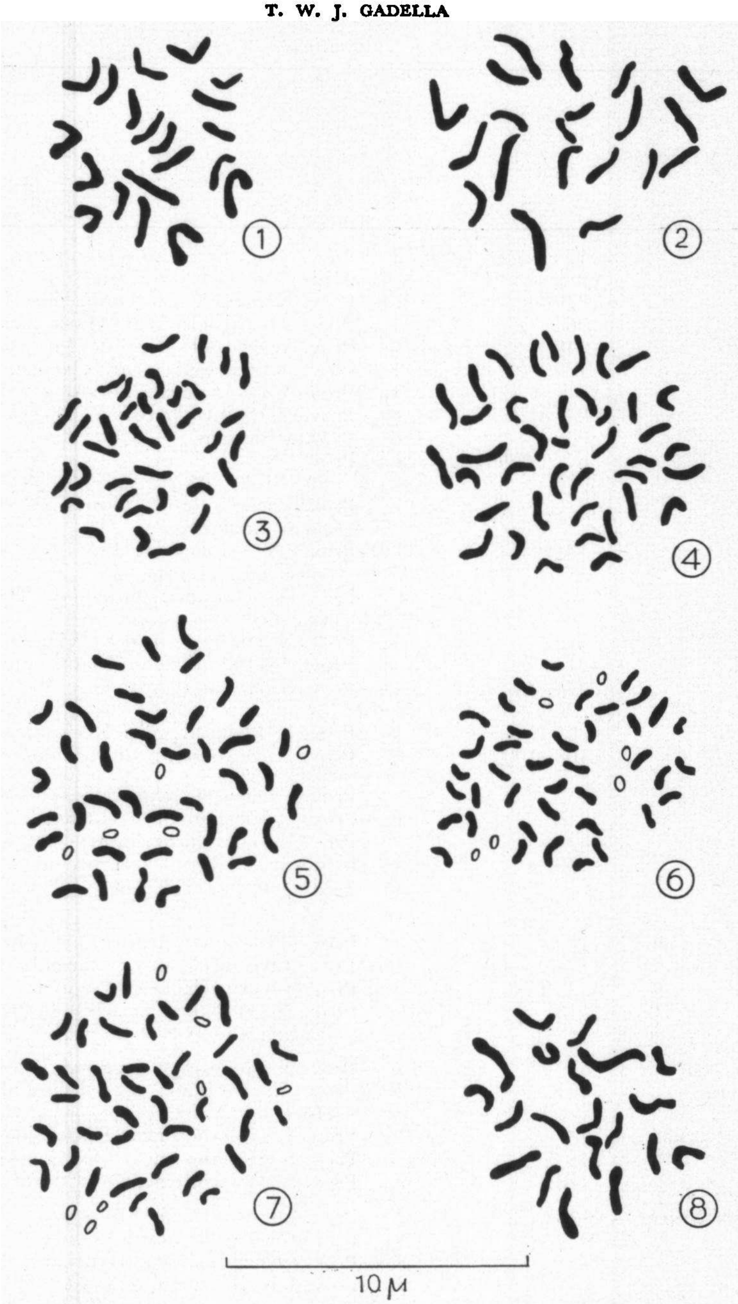


Fig. 1A. 1. *Viola odorata* L. 2n = 20. Orig.: Epen; 2. *Viola hirta* L. 2n = 20. Orig.: Eys; 3. *Viola riviniana* Rchb. 2n = 35. Orig.: Mechelen; 4. *Viola riviniana* Rchb. 2n = 40. Orig.: Rhenen; 5. *Viola riviniana* Rchb. 2n = 45. Orig.: Putten; 6. *Viola riviniana* Rchb. 2n = 46. Orig.: Valkenburg; 7. *Viola riviniana* Rchb. 2n = 47. Orig.: Sleen; 8. *Viola reichenbachiana* Jord. ex Bor. 2n = 20. Orig.: Epen.

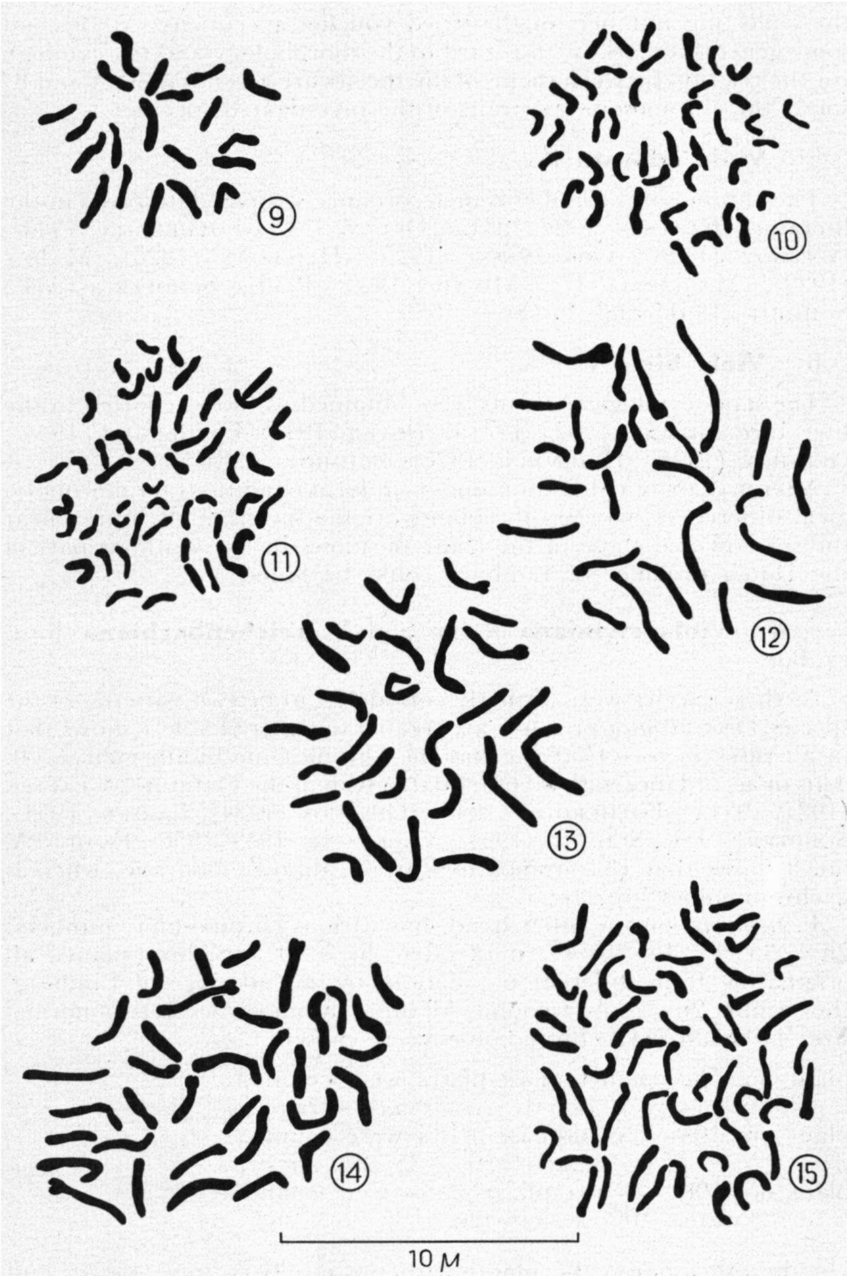


Fig. 1B. 9. *Viola rupestris* F. W. Schmidt var. *arenaria* (D.C.) W. Beckr. 2n = 20. Orig.: Rockanje; 10. *Viola canina* L. 2n = 40. Orig.: Gramsbergen; 11. *Viola palustris* L. 2n = 48. Orig.: Maarn; 12. *Viola tricolor* L. subspec. *curtisii* (Forst.) Syme 2n = 26. Orig.: Oost-Voorne; 13. *Viola tricolor* L. subspec. *tricolor* 2n = 26. Orig.: Hollandse Rading; 14. *Viola arvensis* Murr. 2n = 34. Orig.: Emmen 15. *Viola calaminaria* Lej: 2n = 52. Orig.: la Calamine.

the collecting numbers of the dried voucher specimens. At the end some general remarks with regard to the morphology and the cytology (or the relation between them) of the species are given. Fig. 1 (A and B) shows the chromosome portraits of the investigated species.

a) **Viola odorata** L.

The chromosome number is in accordance with all data given in the literature: Clausen (1927, 1931a), Delay (1948), Fothergill (1944), Gershoy (1928), Gorczynski (1929), Heilborn (1926), Madge (1929), Manch (1937), Miyaji (1929, 1930), Schöfer (1954), Schmidt (1961) and Théron (1939).

b) **Viola hirta** L.

The same cytological results were obtained as those reported in the literature: Clausen (1927, 1931a), Delay (1948), Fothergill (1944), Gershoy (1934), Heilborn (1926), Schmidt (1961).

After 4 years of cultivation under uniform conditions no morphological differences between the plants originating from the dunes near the sea-cost and those of the chalk-meadows in the southern part of the Dutch province of Limburg could be found.

c, d) **Viola riviniana** Rchb. and **V. reichenbachiana** Jord. ex Bor.

As these species were formerly considered to be two varieties of the species *Viola silvatica* Fr., they are treated together. Table 1 shows that in all cases *V. reichenbachiana* has the chromosome number $2n = 20$. This in accordance with all other data given in the literature: Clausen (1927, 1931a), Fothergill (1944), Gershoy (1934), Larsen (1954), Schöfer (1954), Schmidt (1961), Valentine (1949, 1950). Figure 1A, no. 8 shows that 18 chromosomes are of almost equal size, whereas 2 chromosomes are larger.

*V. riviniana*, on the other hand, has various chromosome numbers: $2n = 35, 40, 45, 46, 47$ (most often $2n = 40$). In three plants, all originating from different populations in the province of Limburg, the number $2n = 35$ was found. All these chromosomes were of normal size; no small or B-chromosomes were present.

plant no. 50: 5 metaphase-plates were counted:
$4\times$ exactly $2n = 35$, $1\times$ $2n =$ ca. 33
plant no. 165: 7 metaphase-plates were counted:
$6\times$ exactly $2n = 35$, $1\times$ $2n = 37$.
plant no. 198: 12 metaphase-plates were counted:
$10\times$ exactly $2n = 35$, $2\times$ $2n = 34$.

On the other hand, the plants with the numbers $2n = 45$, 46 and 47 showed 5, 6 or 7 small supernumerary or B-chromosomes. All plants showed quite normal pollengrains.

Notwithstanding the fact that there are many differences between these species, they resemble each other to a certain extent in general

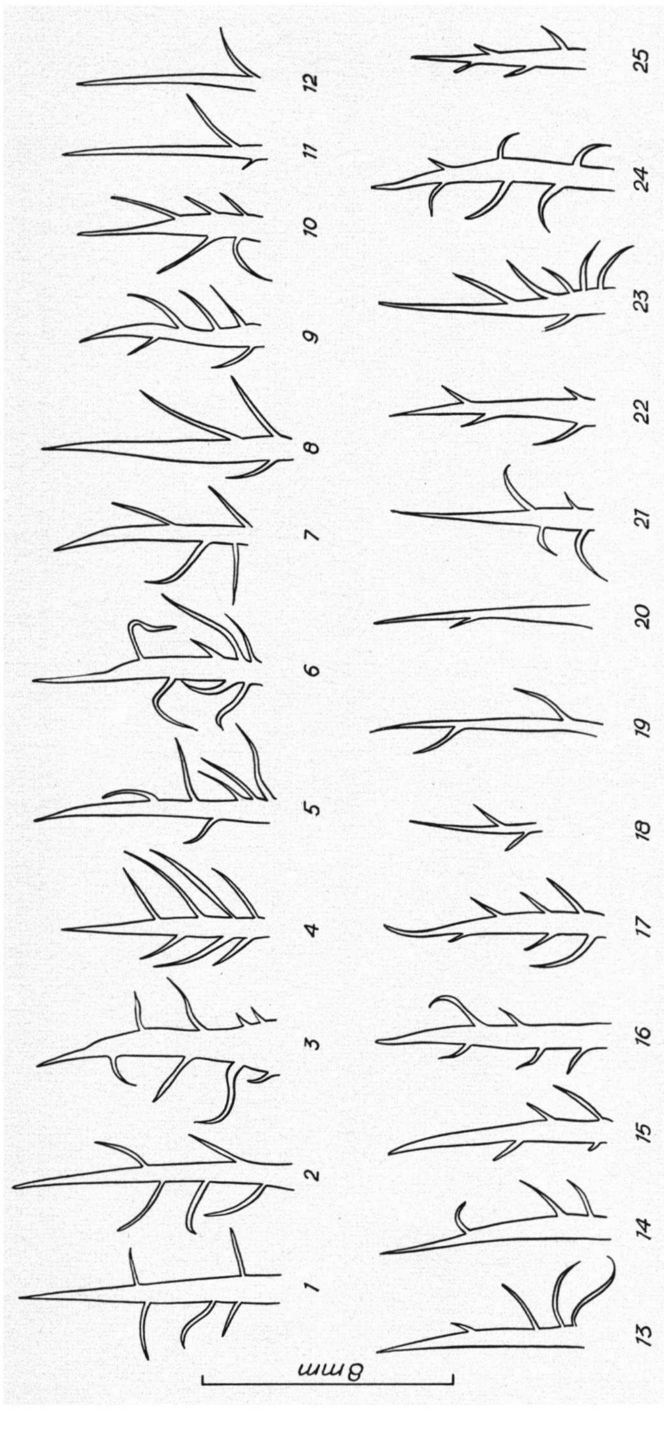


Fig. 2. Variation in the shape of the stipules of *Viola reichenbachiana* Jord. ex Bor. (2n = 20). Orig.: 1–2: Epen; 3–6: Bentheim; 7–10: Rijckholt; 11–17: Eys; 18–20: Gulpen; 21–25: Ootmarsum.

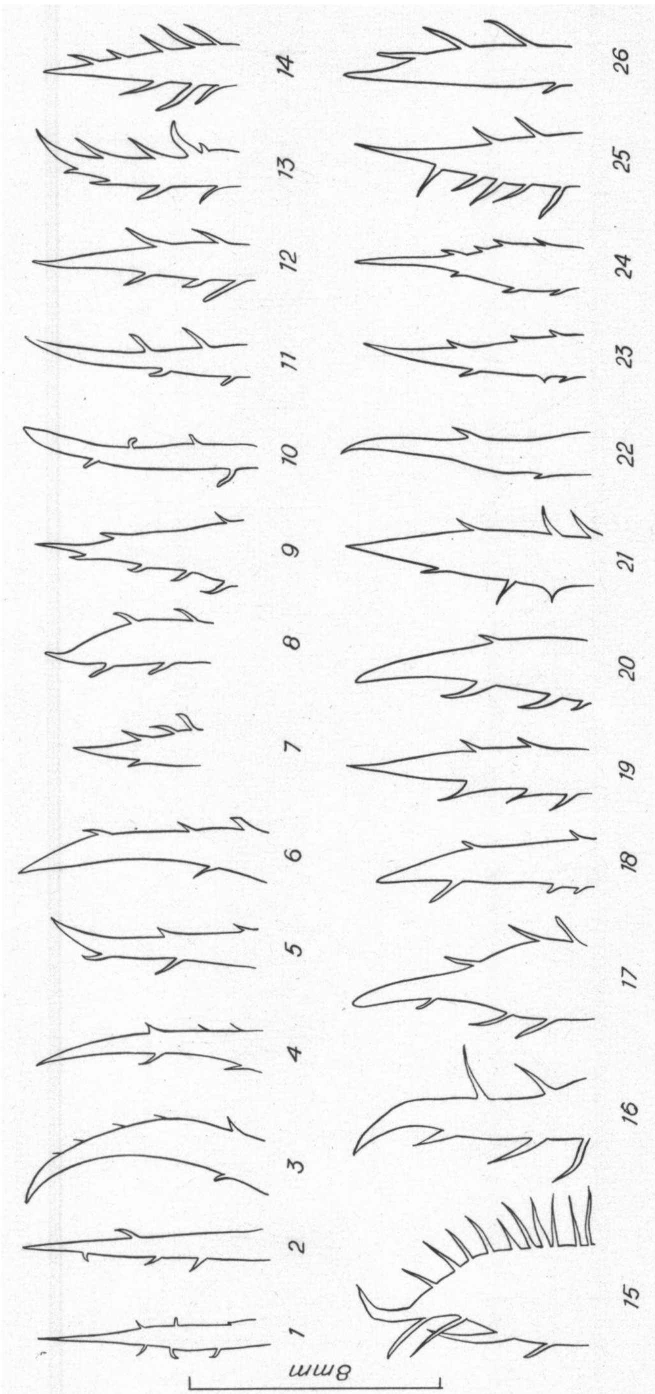


Fig. 3A. Variation in the shape of the stipules of the middle cauline region of *Viola riviniana* Rchb. Origin: 1–2: Lattrop; 3–9: Denekamp; 10–17: Oldenzaal; 18–24: Weerdinge; 25–26: Mook. Chromosome number in all samples: 2n = 40.

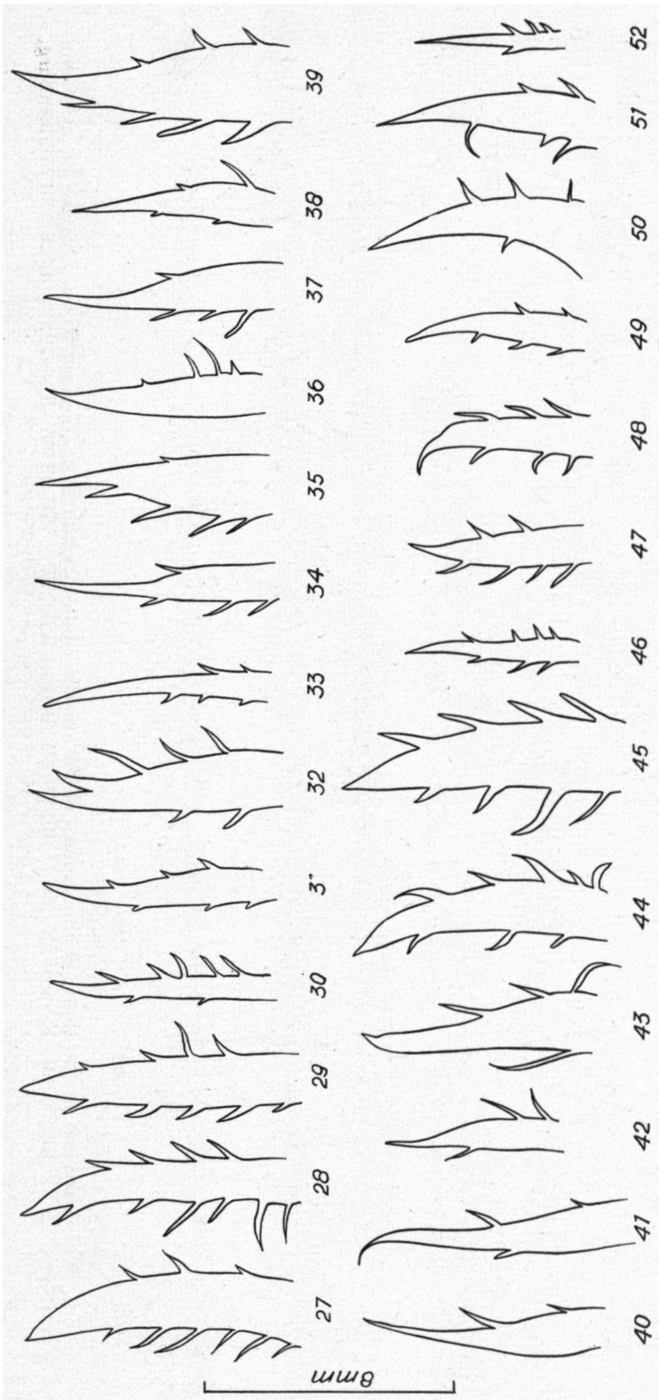


Fig. 3B. Variation in the shape of the stipules of the middle cauline region of *Viola riviniana* Rchb. Origin: 27–29: Mook; 30–32: Berg en Dal; 33–43: Rockanje; 44–45: Bergen aan Zee; 46–49: Rhenen; 50–52: Vijlen; Chromosome number in all samples: $2n = 40$.

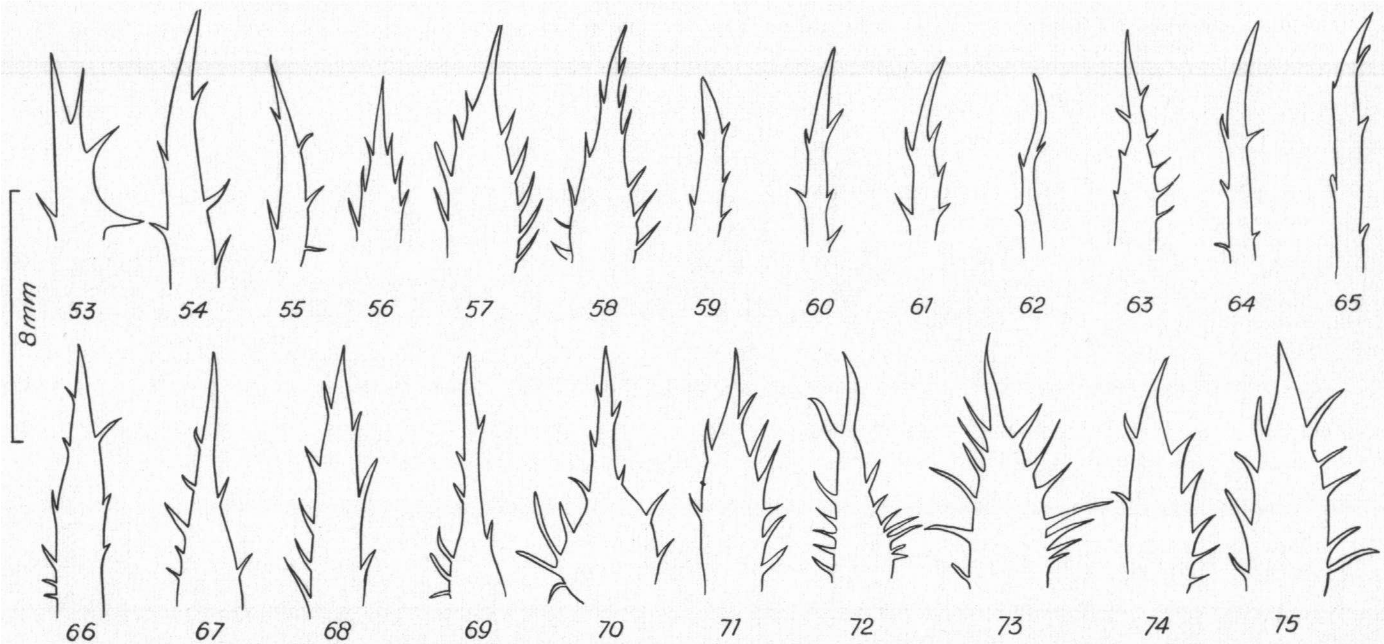


Fig. 3C. Variation in the shape of the stipules of the middle cauline region of *V. riviniana* Rchb. Origin: 53–55: Schin op Geul, 2n = 40; 56–58: Robertville, 2n = 40; 59–62: Bérisménille, 2n = 40; 63–66: Epen, 2n = 45; 67–70: Valkenburg, 2n = 46; 71–75: Sleen, 2n = 47.

appearance. As it is almost impossible to study some flower characters, among which some of diagnostic value, in dried material, the morphological studies are only based on living plants. The characters described below remained constant under uniform garden conditions.

The vegetative characters of both species agree in many respects. The leaves of *V. reichenbachiana* are often smaller than those of *V. riviniana*, but there is a considerable overlap. The same holds true for the hairiness of the leaves. The stipules are manifestly different. Fig. 2 shows the variation in the shape of the stipules of *V. reichenbachiana*, and Fig. 3 (A, B, C) of *V. riviniana*. Stipules of plants originating from different habitats were examined. In every case the most typical examples were drawn. The stipules of the uppermost leaves of *V. riviniana* are often entire, but those of the middle ones are fringed. In *V. riviniana* the fringes are short; the width of a stipule at the place of insertion of a fringe is never smaller than the length of that fringe. In *V. reichenbachiana* the fringes are longer. The number of fringes per stipule fluctuates in both species. In *V. riviniana* the stipules are generally, but not always, broader than in *V. reichenbachiana*. The plants originating from Sleen (no. 180), 2n = 47, have broad stipules. This is, however not typical for all plants with a high chromosome number, for there are also plants with the number 2n = 40 (Fig. 3A, no. 15) with broad stipules. As was pointed out by VALENTINE (1949), the plants with supernumerary chromosomes have adventitious shoots. This phenomenon could only be observed in the plants from Sleen (plant no. 180). It was, however, not checked in all plants.

The flowers of the two species are clearly different. Figure 4 shows the differences between the lower, spurred, petals. All plants with the number 2n = 20 have small petals, the lower one provided with a slender, pointed, dark purple spur, whereas the plants with the number 2n = 35, 40, 45, 46, 47 have broad petals and stouter, furrowed spurs of different colour (white, light to dark purple, and partly white, partly purple.) The venation of the lower petal agrees with that of the British plants described by Valentine. The purple veins of *V. riviniana* branch out and fill the distal part of the petal, whereas those of *V. reichenbachiana* are fewer in number and are restricted to the central part of the petal. The veins of *V. riviniana* remain distinctly seperate, those of *V. reichenbachiana* become diffuse in the usually dark purple-spotted centre of the lower petal. The colour of the spur of *V. riviniana* is not correlated with the occurrence of supernumerary chromosomes: the spur is white in plant, plant no. 131 (2n = 46), plant no. 213 (2n = 45) and purple in plant no. 39 (2n = 45), plant no. 17 (2n = 46), plant no. 243 (2n = 47) and plant no. 180 (2n = 47).

The diameter of the pollengrains is somewhat larger in *V. riviniana* than in *V. reichenbachiana* (table 2).

The number of seeds per fruit is higher in *V. riviniana* than in *V. reichenbachiana*, as shows Fig. 5. Fruits from cleistogamous flowers were collected at random in July in different populations, viz. 86 fruits of *V. reichenbachiana* and 105 of *V. riviniana*.

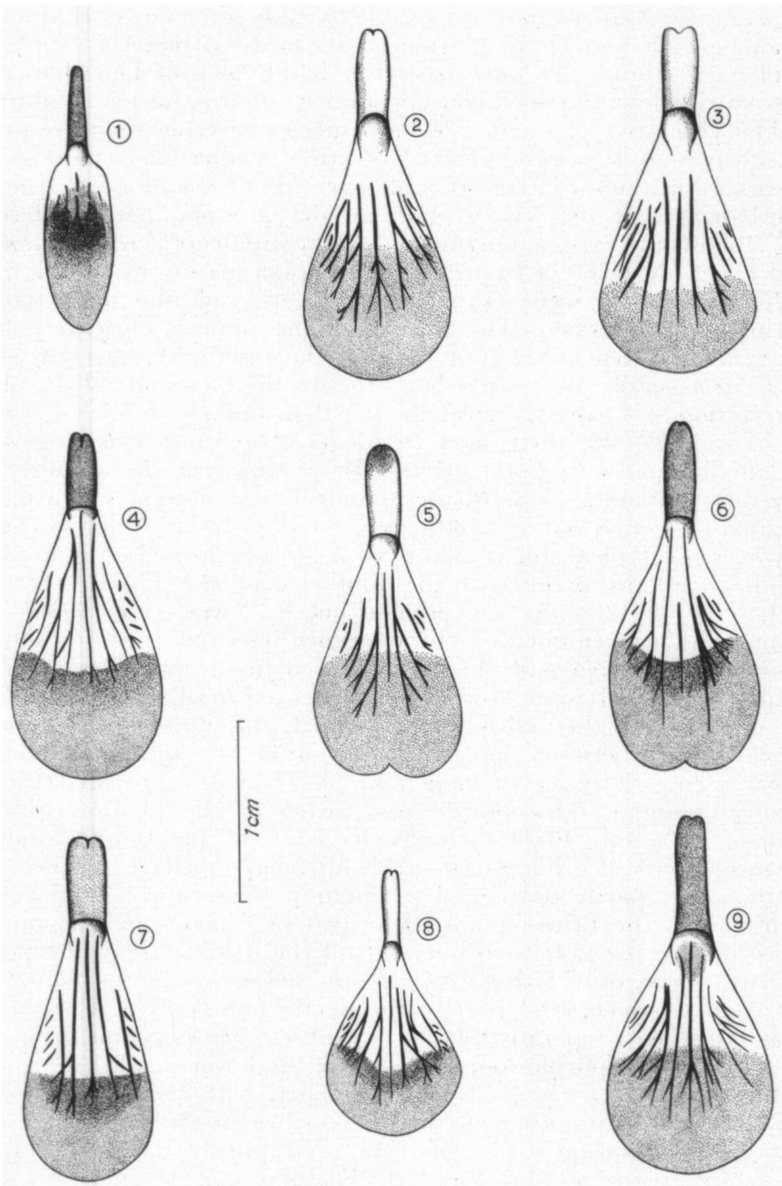


Fig. 4. Lower petal and spur of *Viola reichenbachiana* Jord. ex Bor. (1) and *Viola riviniana* Rchb. (2–9). 1. 2n = 20. Orig.: Ootmarsum. Spur dark purple; 2. 2n = 40. Orig.: Epen. Spur white; 3. 2n = 40. Orig.: Lattrop. Spur white; 4. 2n = 40. Orig.: Oude Mirdum. Spur purple; 5. 2n = 40. Orig.: Rhenen. Spur white-purple; 6. 2n = 40. Orig.: Schin op Geul. Spur purple; 7. 2n = 45. Orig.: Epen. Spur light purple; 8. 2n = 46. Orig.: Oldenzaal. Spur white; 9. 2n = 47. Orig.: Sleen. Spur purple.

TABLE 2

| Species and origin | 2n | 20–24 μ | 24–28 μ | 28–32 μ | 32–36 μ | 36–40 μ | 40–44 μ | 44–46 μ |
|---|---|---|---|---|---|---|---|---|
| V. reichenbachiana (Mechelen) | 20 | 2 % | 48 % | 44 % | 2 % | — | — | — |
| V. reichenbachiana (Ootmarsum) | 20 | 2 % | 39 % | 59 % | — | — | — | — |
| V. reichenbachiana (Bentheim) | 20 | — | 30 % | 70 % | — | — | — | — |
| V. riviniana (Rhenen) | 40 | — | 1 % | 40 % | 59 % | — | — | — |
| V. riviniana (Weerdinge) | 40 | — | 3 % | 57 % | 35 % | 3 % | — | — |
| V. riviniana (Rockanje) | 40 | — | — | 78 % | 22 % | — | — | — |
| V. riviniana (Lattrop) | 40 | — | 8 % | 48 % | 36 % | 6 % | — | — |
| V. riviniana (Valkenburg) | 46 | — | — | 36 % | 38 % | 26 % | — | — |
| V. riviniana (Sleen) | 47 | — | — | 18 % | 48 % | 28 % | 4 % | 2 % |

The relation between the number of chromosomes and the diameter of the pollengrains of 9 populations of *V. riviniana* Rchb. and *V. reichenbachiana* Jord. ex Bor. (In each sample 100 pollengrains were measured).

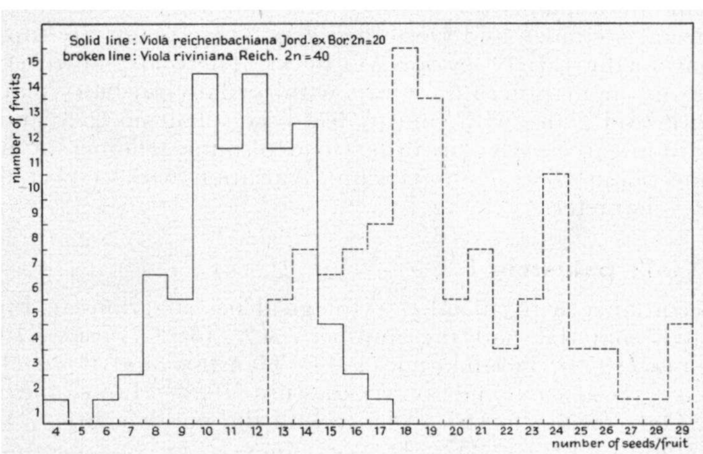


Fig. 5. The difference in the number of seeds per fruit between *Viola riviniana* Rchb. and *Viola reichenbachiana* Jord. ex Bor.

The germination of the seeds presented some difficulties. The seeds only germinated outside the greenhouse after a period of frost. This is in accordance with an observation by GERSHOY, (1928).

Hybrids between the species were not met with in the Netherlands. On the island of Voorne a hybrid between *V. riviniana* and *V. canina* was found. This plant resembled the *V. riviniana* parent, but had much larger vegetative and floral parts. The chromosome number of the hybrid turned out to be 2n = 40. No seeds were formed during 3 years of cultivation, all cleistogamous flowers withered within a few days. The pollengrains were quite abnormal, mostly shrivelled, and many dwarf pollengrains were formed.

e) **Viola rupestris** F. Schmidt var. **arenaria** (D.C.) W. Beck.

The chromosome number 2n = 20 agrees with all cytological observations mentioned in the literature: CLAUSEN (1929, 1931a), GERSHOY (1934), SCHÖFER (1954), SCHMIDT (1961).

f) **Viola canina** L.

In accordance with several authors: BRUUN (1932), FOTHERGILL (1944) GERSHOY (1934), LÖVE and LÖVE (1956), MOORE (1957), MOORE and HARVEY (1961), SCHÖFER (1954), SCHMIDT (1961), but in contrast to J. CLAUSEN (1931b, 1932), for this species only the number 2n = 40 was found in the Netherlands.

Contrary to the observations of J. Clausen in Denmark, in our material no abnormal pollengrains were observed. Morphologically the material from Rosmalen was somewhat different from the plants collected in other parts of the country. These plants were tall and slender, but after 4 years of cultivation under uniform conditions they did not differ from other plants.

In some dune plants the leaf-base was cuneate, not cordate, as in plants from Rosmalen and Oosterhesselen. These plants are supposed to belong to the variety *dunensis* W. Beckr. There are, however, also plants from the margin of the dunes with cordate leaf-base (plant no. 81) and inland plants with cuneate leaf-base (plant no. 214). Further investigations are needed in order to determine whether or not in our dune plants there exists ecoclinic variation with respect to the leaf-base character.

g) **Viola palustris** L.

In accordance with all other cytological investigations in Europe, the Dutch material had the number 2n = 48: CLAUSEN (1931a), FERNANDES (1950), FOTHERGILL (1944), GERSHOY and BOLD (1934), JÖRGENSEN, SÖRENSEN and WESTERGAARD (1958), LÖVE and LÖVE (1956). Only in North Africa plants with the number 2n = 24 have been found (QUEZEL, 1957).

h) **Viola tricolor** L.

The chromosomes of *V. tricolor* L., *V. arvensis* Murr., and *V. calaminaria* Lej. (all belonging to the section *Melanium*) are larger than those of the species dealt with in this paper, which belong to the section *Nominium*.

The inland as well as the dune form has the chromosome number 2n = 26. This is in accordance with all other cytological investigations:

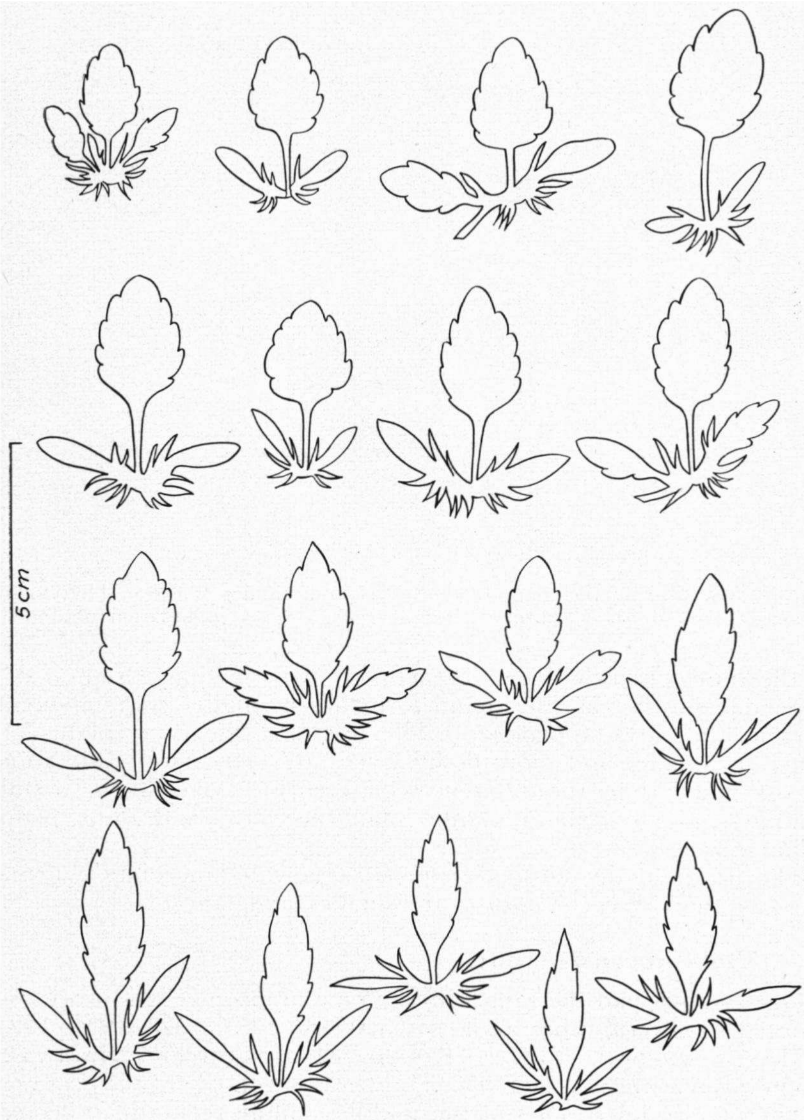


Fig. 6. Variation in the shape of the leaves and stipules of the middle cauline region of *Viola tricolor* L. subspec. *tricolor* (population Hollandse Rading).

Clausen (1921, 1926, 1927, 1931a), Fothergill (1938, 1941, 1944), Gershoy (1934).

Morphologically the two subspecies are clear-cut. During 4 years of cultivation under uniform conditions the annual inland form (subspec. *tricolor*) kept its ascending habit, whereas the perennial dune plants (subspec. *curtisii*) remained prostrate. Besides, the length of the spur

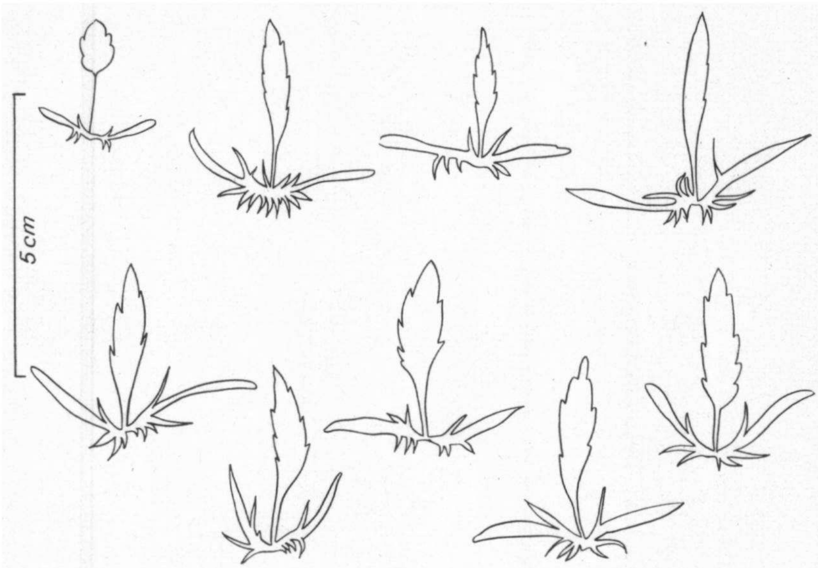


Fig. 7. Variation in the shape of the leaves and stipules of the middle cauline region of *Viola tricolor* L. subspec. *curtisii* (Forst.) Syme (population Ouddorp).

of the dune plants was (and remained) twice as long as the calyx appendages, whereas the inland form has a smaller spur, about as long as the calyx appendages. The leaves of the dune plants are generally smaller and more fleshy, even after 4 years of cultivation. Figs. 6 and 7 show the differences between the leaves of the middle cauline region of inland plants (plant no. 116) and dune plants (plant no. 128).

The leaves of the subspecies *tricolor* are ovate-lanceolate, whereas those of the subspecies *curtisii* are smaller and lanceolate.

### i) **Viola arvensis** Murr.

Our samples had the same chromosome number as was previously counted by several other authors: Clausen (1921, 1922, 1926, 1927, 1931a), Fothergill (1944), Kondo, Matsunami and Hagiwara (1956), Löve and Löve (1956).

The leaves and stipules of the middle cauline region of collection number 216 are shown in Fig. 8, in order to permit a comparison with the leaves of *V. tricolor*. The stipules are sometimes of the same shape as the foliage leaves (fig. 8, no. 2); the leaves are often much larger than those of *Viola tricolor*.

### j) **Viola calaminaria** Lej.

11 plants were collected near La Calamine (Belgium) on rubble of a zinc mine in a typical **Violetum calaminariae**. Six plants permitted chromosome counts: 2n = 52.

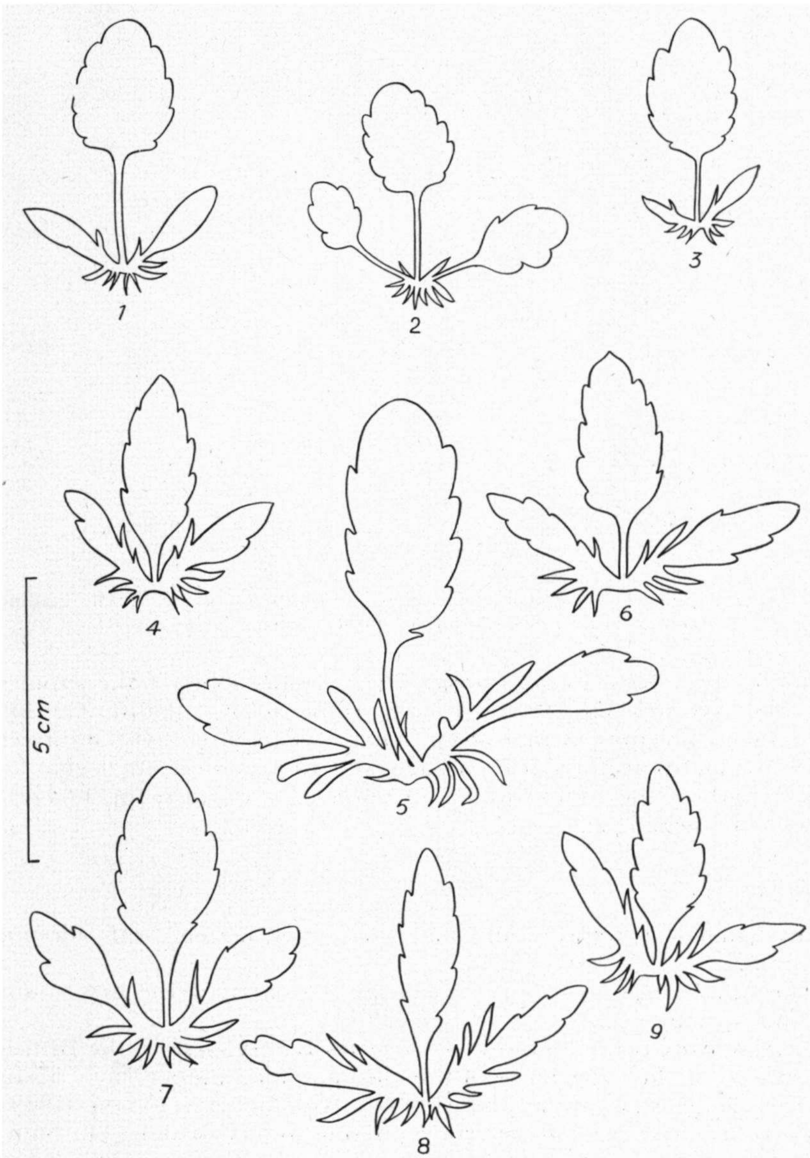


Fig. 8. Variation in the shape of the leaves and stipules of the middle cauline region of *Viola arvensis* Murr. (population Hilversum).

*Viola calaminaria* has many morphological characters in common with the *Viola tricolor*-complex. For example, the length of the spur agrees with that of *V. tricolor.* L. subspec. *curtisii* (Forst.) Syme. Sometimes the stipules are palmatipartite (fig. 9 no. 1) which is characteristic for *V. lutea*, sometimes they are pinnatipartite, which

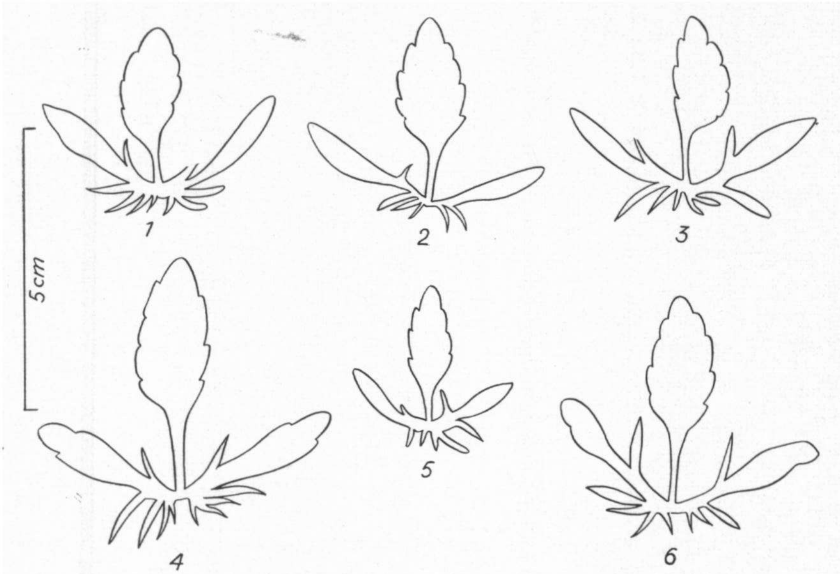


Fig. 9. Variation in the shape of the leaves and stipules of the middle cauline region of *Viola calaminaria* Lej. (population la Calamine).

is said to be typical for *V. tricolor*. The terminal lobes of the stipules may be crenate (Fig. 9 no. 4), according to FOTHERGILL (1944) a character not present in *V. lutea*. After 4 years of cultivation under uniform conditions these leaf characters as well as some other characters (length of the spur, width and shape of the calyx appendages) remained unchanged.

## CONCLUSIONS

In cytological respect our material agrees largely with foreign material.

The number 2n = 52 for *V. calaminaria* represents the first count for this species.

Cytologically our material of *V. riviniana* is very close to the British material. For the occurrence of the chromosome number 2n = 35 in *V. riviniana* no explanation can be offered. Although VALENTINE (1949) reports this number for material originating from Croxdale, Durham, he does not discuss these plants further. The report is perhaps due to a printer's error.

Contrary to Schmidt, Schöfer and Valentine, no hybrids between *V. riviniana* and *V. reichenbachiana* could be found. Plants with 2n = 30 were not met with, even in mixed populations. The plants with the number 2n = 40 showed all very clearly the typical characters of *V. riviniana* as described by Valentine and could, therefore, not be regarded as hybrids. Moreover, all the plants examined had a good seed production, which does not occur in artificially produced hybrids.

On the other hand, in South Germany the seed production of the plants regarded as hybrids with the numbers 2n = 30 and 2n = 40 was not abnormal. It is to be regretted that Schöfer failed to give detailed descriptions of the putative hybrids. In the author's opinion there are two possibilities concerning these plants:

1) the material from S. Germany is genetically different from the English material;
2) the plants with the number 2n = 40 are not of hybrid origin.

The absence of supernumerary chromosomes in the material from S. Germany corroborates the first assumption. The production of normal seeds is in better agreeance with the second. It would be highly desirable to have detailed descriptions of some of the hybrid populations in S. Germany, in order to facilitate a comparison with the material from England and the Netherlands.

The Dutch material of *V. riviniana* is morphologically variable. It was, however, not possible to divide the plants from 11 different populations into the subspecies *minor* and *nemorosa*, even after 4 years of cultivation under uniform garden conditions. Some plants from woods had 3 *nemorosa* and 5 *minor* characters, while other plants from the open dune landscape showed 4 *nemorosa* and 4 *minor* characters. There was no relation between the environment and morphological characters, nor did any plant possess a combination of the characters regarded as typical for the subspecies *nemorosa* and *minor* by Valentine. Therefore it seems impossible to divide the Dutch material into these two subspecies. VALENTINE (1941) suggests that plants of the *nemorosa* type may have more than 40 chromosomes, whereas the plants of the *minor* type always have 40 chromosomes. The Dutch plants with supernumerary chromosomes however, showed several *minor* characters.

Heimans's opinion that *V. calaminaria* should not be referred to *V. lutea* but to the *V. tricolor*-complex could be confirmed. KLOOS (1924) was already of the opinion that *V. calaminaria* is related to *V. tricolor*. Heimans concludes on several grounds that *V. calaminaria* could be derived from *V. tricolor* L. var. *alpestris* Ging. apud DC. [= *V. alpestris* (DC.) Wittr.]

The subspecies *curtisii* of *V. tricolor* resembles *V. calaminaria* in many respects. The number of chromosomes and the ecological requirements are in favour of treating *V. calaminaria* as a separate species.

Contrary to *V. riviniana* no intraspecific cytological variation could be observed in *V. canina* in the Netherlands. Only in Denmark fertile plants (some of which are hybrids) with more than 40 chromosomes are present.

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## REFERENCES


BRUUN, H. G. 1932. A theory on the cytologically irregular species Viola canina. Hereditas **16**: 63–72.

CLAUSEN, J. 1921. Studies on the collective species Viola tricolor L. Bot. Tidsskr. **37**: 204–221.

————. 1922. Studies on the collective species Viola tricolor L. (II). Bot. Tidsskr. **37**: 363–461.

————. 1926. Genetical and cytological investigations on Viola tricolor L. and V. arvensis Murr. Hereditas **8**: 1–156.

————. 1927. Chromosome number and the relationship of species in the genus Viola. Ann. of Bot. **41**: 677–714.

————. 1929. Chromosome number and relationship of some North American species of Viola. Ann. of Bot. **43**: 741–764.

————. 1931a. Danmarks Viol-Arter. Bot. Tidsskrift **41**: 317–335.

————. 1931b. Viola canina L., a cytological irregular species. Hereditas **15**: 67–88.

————. 1931c. Cytogenetic and taxonomic investigations on Melanium violets. Hereditas **15**: 219–308.

————. 1932. Remarks upon H. G. Bruun's paper on Viola canina L. Hereditas **17**: 67–70.

DELAY, C. 1948. Recherches sur la structure des noyaux quiescents chez les phanérogames. Rev. de Cytol. et de Biol. Vég. **10**: 103–229.

FERNANDES, A. 1950. Sobre a cariologia de algumas plantas da Serra do Gerês. Agron. Lusit. **12**: 551–600.

FOTHERGILL, P. G. 1938. Studies in Viola. I. The cytology of a naturally occurring population of hybrids between V. tricolor and V. lutea Huds. Genetica **20**: 159–186.

————. 1941. Studies in Viola III. An account of the inheritance of certain characters in the progeny of plants of a wild population of Viola hybrids. New Phytol. **40**: 139–151.

————. 1944. Studies in Viola IV. The somatic cytology and taxonomy of our British species of the genus Viola. New Phytol. **43**: 23–35.

GERSHOY, A. 1928. Studies in North American Violets. I. General considerations. Vermont Agric. Exp. Stat. Bull. **279**: 1–18.

————. 1934. Studies in North American Violets. III. Chromosome numbers and species characters. Univ. Vermont Agric. Exp. Stat. Bull. **367**: 1–92.

———— and H. C. BOLD. 1934. Studies in North American Violets IV. Univ. Vermont Agric. Exp. Stat. **378**: 1–35.

GORCZYNSKI, A. 1929. Recherches histo-cytologiques sur les fleurs cleistogames chez Lamium amplexicaule, Oxalis acetosella et Viola odorata. Acta Soc. Bot. Polon. **6**: 248–295.

GRIESINGER, R. 1937. Ueber hypo- und hyperdiploide Formen von Petunia, Hyoscyamus, Lamium und einige andere Chromosomenzählungen. Ber. Deutsch. Bot. Ges. **55**: 556–571.

HEIMANS, J. 1936. De herkomst van de zink-flora aan de Geul. Nederl. Kruidk. Arch. **46**: 878–893.

————. 1960. Taxonomic, phytogeografical and ecological problems round Viola calaminaria, the zinc violet. Publ. Natuurhist. Genootsch. Limburg **12**: 55–71.

HEILBORN, O. 1926. Bidrag till Violaceernas cytologi. Sv. Bot. Tidskr. **20**: 414–419.

JÖRGENSEN, C. A., TH. SÖRENSEN and M. WESTERGAARD. 1958. The flowering plants of Greenland. A taxonomical and cytological survey. Dansk Vid. Selsk. Biol. Skr. **9**, 4: 1–172.

KLOOS, A. W. 1924. Het geslacht Viola in Nederland. Nederlands Kruidk. Arch. **34**: 138–208.

KONDO, N., M. MATSUNAMI, and T. HAGIWARA. 1956. Chromosome number of pansy and some cultivated Viola. Jap. J. Genet. **31**: 302.

LARSEN, K. 1954. Chromosome numbers of some European flowering plants. Bot. Tidsskr. **50**: 163–174.

Löve, A. 1950. Some innovations and nomenclatural suggestions in the Icelandic flora.
Bot. Not. (1950): 24–60.

Löve, A. and D. Löve. 1956. Conspectus of the Icelandic Flora. Acta Horti Gotoburgensis **20** (4): 65–291.

Madge, M. A. 1929. Spermatogenesis and fertilization in the cleistogamous flower of Viola odorata L. var. praecox Greg. Ann. Bot. **43**: 545–577.

Manch, D. 1937. Meiosis in an F1 Viola hybrid and its recipocal. Am. Journ. Bot. **24**: 678–683.

Miyaji, Y. 1929. Studien über die Zahlenverhältnisse der Chromosomen bei der Gattung Viola. Cytologia **1**: 28–58.

Miyaji, Y. 1930. Betrachtungen über die Chromosomenzahlen von Violen, Violaceen und verwandten Familien. Planta **11**: 631–649.

Moore, D. M. 1957. The status of Viola lactea. in: Lousley, J. E. (ed). Progress in the study of the British flora. 1957: 97–102.

Moore, D. M. and M. J. Harvey. 1961. Cytogenetic relationships of Viola lactea Sm. and other West European arosulate violets. New Phytol. **60**: 85–94.

Quezel, P. 1957. Peuplement végétal des hautes montagnes de l'Afrique du Nord. Encycl. Biogeogr. et Écol. **10**: 1–445.

Schöfer, G. 1954. Untersuchungen über die Polymorphie einheimischer Veilchen. Planta **43**: 537–565.

Schmidt, A. 1961. Zytotaxonomische Untersuchungen an europäischen Viola-Arten der Sektion Nominium. Oesterr. Bot. Zeitschr. **108**: 20–88.

Théron, A. 1939. Recherches morphologiques et cytologiques sur les fleurs de Viola odorata. Rev. Cyt. Cytophysiol. Vég. **4**: 101–118.

Valentine, D. H. 1941. Variation in Viola riviniana Rchb. New Phytol. **40**: 189–209.

———. 1949. Vegetative and cytological variation in Viola riviniana Rchb. In Willmott, Brit. Flow. Plants and Modern Syst. Methods. (B.S.B.I. Conference 1948): 48–53.

———. 1950. The experimental taxonomy of two species of Viola. New Phytol. **49**: 193–212.

———. 1956. Variation and polymorphism in Viola. Proc. Royal Soc. **145**: 315–319.

———. 1958. Cytotaxonomy of the Rostrate Violets. Proc. Linn. Soc. London **169**: 132–134.

West, J. 1930. Cleistogamy in Viola riviniana with especial reference to its cytological aspects. Ann. Bot. **44**: 87–109.