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A deviating cytotype of *Viola riviniana* from Finland

Harri Harmaja

Botanical Museum, Finnish Museum of Natural History, P.O. Box 7, FIN-00014 University of Helsinki, Finland (e-mail: harri.harmaja@helsinki.fi.)

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The chromosome numbers of five collections of *Viola riviniana* (Violaceae) from South Finland were examined from mitoses in root tip cells. The collections consist of one plant each and originate from five different localities. The plants were subjected to cytological study as they appeared slightly deviating from typical *V. riviniana* (mostly 2n = 40) by possessing some traits (fairly pale, narrow petals and short calyx appendages) reminding of the closely related *V. reichenbachiana* (2n = 20). Four of the plants unexpectedly turned out to have 2n = 30 while one had the expected number 2n = 40. 2n = 30 is an unknown somatic number at least in this group of violets but would suggest F_1 generation hybrids between *V. reichenbachiana* and *V. riviniana*. However, (i) the 2n = 30 plants of the present study are fertile though the hybrid mentioned is known to be highly infertile, (ii) their stipules are not intermediate (but of a kind of their own), and (iii) *V. reichenbachiana* is unknown in the study area. As a conclusion: the plants that deviate from typical *V. riviniana* both morphologically and cytologically may in some way result from hybridisation between *V. reichenbachiana* and *V. riviniana* or they may represent an unknown (almost) cryptic taxon. Previously, fertile plants of this group with 2n = 30 have once been reported from Germany; they were supposed to represent the hybrid between the two species mentioned. A mention is also made about the ploidy level of the 2n = 30 genome: according to current view it represents the triploid level but there is a possibility that the hexaploid level is concerned instead.

Key words: chromosome number, cytology, introgression, *Viola*

Introduction

Viola riviniana (Violaceae) belongs to sect. *Viola* subsect. *Rostratae* and possesses a European distribution area. Variability has been recorded in its vegetative and floral parts (e.g., Valentine 1941, 1950, Gadella 1963, Valentine *et al*. 1968, Jalas 1980, Marcussen *et al*. 2001). The variability of *V. riviniana* can also be seen e.g. in the herbarium material of the Botanical Museum of the Finnish Museum of Natural History (H) though the important original petal colour is unfortunately seldom preserved in the dried condition. During field trips in southern Finland, especially in the 1980s, I noticed that distinct variability in the morphological traits holds true; different-looking specimens were mainly collected from Espoo, Lammi, Lohja, and Siuntio. The following characters, in particular, display variability in the southern Finnish material of *V.*

Fig. 1. *Viola riviniana* (var. ?) *in situ*, Finland, Varsinais-Suomi, Lohja, Maksjoki, 2.VI.1987. A flower of the same plant in side view (left-hand side photo) and in front view (right-hand side photo). An adjacent, similar plant of the same stand yielded 2n = ca. 30. Photo Mauri Korhonen.

riviniana according to my personal observations: the width of the wings in the angles in the stem, the indumentum of the stem and pedicels (both are usually glabrous), the size and relative width of the leaves, the thickness of the leaves, the anthocyanin coloration of the leaf undersides, the size of the stipules and the length of their ciliae, the size of the flowers, the relative width of the petals, the exact tinge and intensity of the petal colour, the venation of the petals, the presence of indumentum in the upper (mostly absent) and the lateral (mostly present) petals, the length, width, shape and colour of the spur, and the length of the calyx appendages. The variability of *V. riviniana* in Finland may partly result from ancient introgressive hybridisation between the closely related, essentially European *V. reichenbachiana* and *V. riviniana* in which traits of the former were infiltrated into the latter. Such introgression is happening in central Europe all the time (Schmidt 1961, Marcussen *et al*. 2001). This theory provides that *V. reichenbachiana* occurred widely in southern Finland during the Holocene.

Some plants (like that in Fig. 1) especially aroused my interest as they displayed some resemblance to *Viola reichenbachiana*. In other words, they differed from typical *V. riviniana* in having smaller corolla, relatively narrower petals that do not overlap and are paler, a more slender and only indistinctly grooved and usually coloured spur. The leaves are generally slightly smaller and slightly more elongated. However, the seed number per capsule, which character was checked in some plants, is high as in *V. riviniana* (roughly twenty), in concordance with Gadella (1963). The stipules, however, are generally somewhat of a type of their own as being small and having only a few, short ciliae. One plant (Uusimaa, Siuntio, Kokkila, 21.VI.1987 *H. Harmaja*), seemingly belonging to this type of *V. riviniana*, was most striking as its flower displayed an atavism: it was radiate with all its petals alike, being simple and narrow, and with leaf-like sepals that slightly exceeded the petals.

Plants that are intermediate between *Viola reichenbachiana* and *V. riviniana* but do not appear to be hybrids, have sometimes been called *V. riviniana* var. *nemorosa*, *V. riviniana* f. *intermedia*, or *V. vicina* in the older literature.

The true *Viola reichenbachiana*, confirmed through chromosome counts, was only fairly recently reported from SW Finland (Stork 1970, 1971) but is still unknown in the rest of the country, including my study area.

The chromosome numbers of the species mentioned are different: *Viola riviniana* has generally 2n = 40 (representing the tetraploid level according to current view) while *V. reichenbachiana* is diploid having 2n = 20 (Valentine 1949, Valentine 1950, Schöfer 1954, Schmidt 1961,

Gadella 1963, Valentine *et al*. 1968, Stork 1971, counts cited by Löve & Löve 1975, Lövkvist & Hultgård 1999, Marcussen *et al*. 2001). Quite recently, a new species belonging to this group, *V. laricicola* with 2n = 20, was described from the European Alps (Marcussen 2003). Moreover, the numbers 2n = 35, 45, 46, and 47 occur very occasionally in *V. riviniana*, the latter three numbers being due to the presence of small B chromosomes (Valentine 1949, 1950, Gadella 1963). Therefore, I checked the chromosome numbers of some morphologically deviating *V. riviniana* plants, described above, to see if a cytological analysis could give some clarification to their taxonomic position. The fertility of the analysed plants was checked. In addition, some effort was also made to examine the size of the pollen grains of the deviating *V. riviniana* plants.

Materials and methods

Flowering plants of *Viola riviniana* like that in Fig. 1, deviating from the typical representatives of this species in the way described above, were collected in early summer of the years 1987 and 1988 from seven different localities in South Finland (Lohja in bioclimatical province of Varsinais-Suomi, Siuntio in prov. Uusimaa, and Lammi in prov. Etelä-Häme). Each of the seven samples consisted of one plant individual. The plants were transferred indoors where they were kept in vases with water in order to get fresh roots developed. In the same year when collected, the somatic (2n) chromosome number for each plant was determined on mitoses in the root tip cells, following the procedures described by Jalas and Pellinen (1985). The work was successful in five cases and the collecting data, the respective chromosome numbers, and some other information of those five plants are listed below in Results. The counting of the chromosomes failed in two cases (one plant originated from Lohja, the other from Lammi). The pollen quality of two cytologically studied plants was examined: both were observed under the dissecting microscope and one of them also under the light microscope; for staining cotton blue (methyl blue) in lactic acid was used. Three of the voucher plants were allowed to grow till the development of the capsules and their seeds could be observed. All plants that were examined were preserved as voucher specimens; these are in my herbarium and will be deposited in H. In addition, some plants (unlisted; not studied cytologically) of typical *V. riviniana*, of the deviating *V. riviniana* (both from southern Finland) as well as of *V. reichenbachiana* (from SW Finland and Sweden) were examined for comparison as to the quality and size of pollen (under the light microscope, with cotton blue staining), the development of capsules, and the development and the number of seeds per capsule.

Results

Voucher specimens (individuals) of *Viola riviniana* with their respective somatic chromosome numbers:

1. 2n = ca. 30. Lohja, Maksjoki, marked when flowering and collected on 14.VI.1987 *H. Harmaja*. Capsules well developed; two of them were examined as to the seed number: they contained 19 and 26 seeds, respectively. Infected by the rust *Puccinia violae* (II).
2. 2n = ca. 30. Lohja, Hermala, Kalkkimäki, collected when flowering on 21.VI.1987 *H. Harmaja*. Capsule small and seeds few but both otherwise normal.
3. 2n = ca. 30. Lohja, Jalassaari, Heimo, collected in flower on 2.VI.1988 *H. Harmaja & Timo Harmaja*. Pollen appeared good under the dissecting microscope. This record must be considered uncertain as only one metaphase plate was good enough for counting the chromosomes.
4. 2n = 30. Siuntio, Kokkila, collected in flower on 2.VI.1988 *H. Harmaja & Timo Harmaja*. Pollen appeared good under the dissecting microscope. The grains proved to be predominantly normal and stain well when examined in cotton blue, being 40–45 μm long. The stomata of the underside epidermis of the leaves were 25–32 μm long. Infected by *Puccinia violae* (I).
5. 2n = ca. 40. Lammi, Ylänäinen, Pilnäistenkalliot, collected when flowering on 29.V.1987 *H. Harmaja*. Capsules well developed, one of them with 22 good seeds.

Discussion

Four of the five plants that were found deviating in the field and of which the chromosome number was obtained, yielded the unexpected number 2n = 30, i.e., an apparently triploid number in this group of violets. One plant yielded 2n = ca. 40, i.e., the normal number of *Viola riviniana*. This last-named plant deviated less than the others as its petals were not paler than normally in *V. riviniana* though the spur was violet and the calyx appendages were short. The main results are remarkable as four plant individuals yielded 2n = 30 which would appear a new number in *V. riviniana* and probably in the whole genus (excepting hybrids). However, Schöfer (1954) reported from South Germany putative hybrids between *V. reichenbachiana* and *V. riviniana*, stated to be fertile (!), with 2n = 30.

In the following, I discuss three alternative explanations for the present cytological observations.

(i) 2n = 30 would appear to refer to F_1 hybrid *Viola reichenbachiana* × *riviniana* which is known from some places in Europe (*see* e.g. Valentine *et al*. 1968) and which indeed has been reported to possess this intermediate number (Schmidt 1961). Likewise, some traits in the morphology of the 2n = 30 plants appear intermediate. However, (a) the voucher specimens were fertile; either the pollen quality or the development of capsules and seeds were analyzed (*see* above); Schmidt (1961) and Valentine *et al*. (1968) state the above hybrid to be highly infertile (however, *see* below for another view), (b) the stipules of the 2n = 30 plants are not intermediate but a bit of a kind of their own (*see* "Introduction"), i.e., differing from those of both of the supposed parents, and (c) in Finland, *V. reichenbachiana* is only known from the Åland islands, being unknown in the mainland, i.e., in my study area.

(ii) An explanation is that the deviating plants result from introgression from *Viola reichenbachiana* towards *V. riviniana*. Such introgression appears probable on cytological and molecular evidence in Central Europe (Schmidt 1961, Marcussen *et al*. 2001). When parents have clearly different chromosome numbers, a wide variety of intermediate chromosome numbers would be expected in different plant individuals of the offspring in case of a massive introgressive hybridization with repeated backcrossings. Such has been proved concerning *Viola epipsila* and *V. palustris* (sect. *Viola* subsect. *Plagiostigma*) in Finland (Sorsa 1965, 1968); the rather highly fertile hybrid swarm, or nothospecies that has developed, is named *V.* × *ruprechtiana*. If the present case indeed could be explained in an analogical way, the possibly established chromosome number (2n = 30) and high fertility (*see* above) would mean that it concerns a fairly well fixed nothospecies which arose from the hybridisation between *V. reichenbachiana* and *V. riviniana*. On the other hand, Schöfer (1954) and Schmidt (1961) found that a spontane hybridization between these two species soon results in a swarm of hybrids and backcrossed plants that predominantly have 2n = 40. Valentine *et al*. (1968) suppose that introgression may also occur from *V. riviniana* towards *V. mirabilis* (likewise of subsect. *Rostratae*, with 2n = 20). Considering the 2n = 30 plants detected in the present study, the problem is that, as stated above, *V. reichenbachiana* is unknown in the whole mainland of Finland, including the Lohja area from where these plants originate. Either that species occurs there but has not yet been discovered or it has occurred there earlier (when warmer climates prevailed during the Holocene) but has later experienced extinction. Anyway, the Lohja area is famous for hosting a rich flora of southern species.

(iii) The hitherto known chromosome numbers of the species of this species group of *Viola* (subsect. *Rostratae*) are 2n = almost always either 40 or 20, never 30 in pure species (e.g., Gadella 1963, Valentine *et al*. 1968). Thus, at the first sight, it would appear very improbable that there would exist a non-hybrid taxon with 2n = 30. If this were the case, a previously unknown (almost) cryptic taxon would be concerned. However, the normal fertility of the voucher specimens (*see* their descriptions) would actually point towards such an explanation. As mentioned above, I also studied for comparison the pollen quality and length of the grains in a few plants of typical *V. riviniana*, deviating *V. riviniana* and *V. reichenbachiana* that were not subject to cytological analysis. The pollen proved to be good in

plants examined. The length of the pollen grains proved to be about 38–50 μm in typical *V. riviniana*, being somewhat smaller, about 35–42 μm in length in *V. reichenbachiana* and one deviating *V. riviniana* from Lohja (Osuniemi). The plant from Siuntio (Kokkila) with 2n = 30 had well-developed grains measuring 40–45 μm. Gadella (1963) likewise found the grains of *V. riviniana* to be larger than those of *V. reichenbachiana* but the measures he gave for both are smaller than mine; that author did not inform in which mountant the pollen were measured. My measurements are too scanty to be statistically significant but the above would suggest that the pollen grains of the deviating *V. riviniana* of South Finland may be slightly smaller than those of typical *V. riviniana*. Interestingly, in the Lohja Maksjoki locality some plants that grew among typical *V. riviniana* (with good pollen) and the deviating pale-flowered *V. riviniana* (one of which had 2n = ca. 30, with good capsules and seeds) appeared intermediate. One of these intermediate appearing plants (specimen collected by H. Harmaja & Timo Harmaja 1.VI.1987) was examined microscopically and it turned out to have poorly developed pollen grains.

Of the above alternatives, the explanation (iii) appears the most plausible and (i) the least plausible one. The present Finnish 2n = 30 plants might thus be considered to represent, if not a new taxon, at least a new cytotype of *Viola riviniana*. The putative hybrids between *V. reichenbachiana* and *V. riviniana*, with 2n = 30 and roughly normal fertility, from South Germany (Schöfer 1954) may actually represent this same cytotype. An analysis of the meiosis and molecular studies, in particular, are urgently needed to solve this problem.

The present case is interesting also because it leads one to question which is the true basic chromosome number (that of the basic genome) in this species group: is it n = 10 as believed hitherto or n = 5? In the latter case, these deviating Lohja plants should be interpreted as hexaploids instead of triploids.

Lastly, a note on additional plants of the present group that need further study. In the spring of 1998, on May 28 and 30, respectively, I found in the inland of southern Finland (Lammi, Jahkola, near Patasuppa), on fertile soils in a glaciofluvial delta, two deviating stands of *Viola riviniana* which are worth mentioning here. These plants displayed traits reminiscent of *V. reichenbachiana* perhaps even more than the 2n = 30 plants of the present study, as also the ciliae of the stipules were fairly long. The cytology, pollen or seed production of these plants have not yet been studied.

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