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# CARYOLOGICAL AND MORPHOLOGICAL INVESTIGATIONS ON A NEW ZINC VIOLET (Cave del Predil, Western Julian Alps, NE-Italy)

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**Keywords:** *Viola calaminaria, Viola tricolor* subsp. *subalpina, Viola tricolor* subsp. *subalpina* var. *raiblensis* var. nova. Adaptation, chromosomes, evolution.

**Abstract:** Caryological and morphological analyses were conducted on *Viola tricolor* subsp. *subalpina* and on a zinc violet from the western Julian Alps, described as *Viola tricolor* subsp. *subalpina* var. *raiblensis* var. nova. Both taxa, which have different ecology and distribution, have 2n = 26 chromosomes. Caryotypic evolution in the zinc violet mainly involves changes in absolute chromosome size and in centromeric and relative size simmetry in respect with var. *subalpina,* from which it appears to have evolved.

## Introduction.

In the course of ecophysiological and vegetational researches concerning plants growing on soils with high zinc and lead content, we had to face the problem of the taxonomical status of a yellow-blue zinc violet belonging to the *Viola tricolor* - complex, which occurs in the mining district of "Miniera di Raibl" in the locality "Cave del Predil", on the western Julian Alps.

In northwestern Europe there are two endemic zinc violets of this complex: one, with yellow flowers, is in eastern Belgium *(Viola calaminaria* (DC.) Lej.), the other, with blue flowers, in western Germany *(V. calaminaria* subsp. *westfalica* (Lej.) Ernst); both grow on zinc and lead mine tailings (Ernst, 1968; Kakes, 1977). The most striking difference between these two taxa is the colour of the flower. The other differences are slight, of quantitative type, and not always mantained in culture.

The zinc violet which occurs on mine tailings inside the "Miniera di Raibl" has morphological characters indicating a close affinity with *V. tricolor* L. subsp. *subalpina* Gaudin; this taxon has a broad distribution in the Alps and is highly variable.

The main aim of this study is to investigate the morphological and caryological differences between zinc violet populations of "Cave del Predil" and wild populations of *V. tricolor* subsp. *subalpina,* which grow on natural soils without high

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concentrations of heavy metals. Another object is to study the relationships among the alpine zinc violet and those of northern Europe.

**Material and Methods**

The morphological and caryological investigations have been carried out on plants collected in the following localities: Cave del Predil (Tarvisio, Udine - Alpi Giulie), inside the mining district of "Miniera di Raibl" at 920 m and Sauris di Sotto (Ampezzo, Udine - Alpi Carniche) at 1080 m.

The morphological investigations, both on fresh and dried material, have been carried out also on exsiccata present in the herbaria of the University of Trieste (TSB) *(V. tricolor* subsp. *subalpina)* and of Liège (LG) *(V. calaminaria).* For the morphological comparison the following characters have been selected: 1) shape of the leaves and stipules of the middle cauline region; 2) size of the flowers; 3) length of the spur related to the calycine appendages; 4) length, width of the seeds and their ratio. The quantitative analysis of the seeds was made on 25 random samples for each taxon. The nomenclature follows Valentine et al. (1968).

The caryological studies were made from mitoses in root tips obtained from seeds, pretreated with 8 - hydroxiquinoline, fixed in Carnoy's fluid, hydrolyzed in 1NHCl and stained with Feulgen method. Chromosome plates were obtained by squash technique; 25 metaphase plates for each taxon were investigated. The caryotypes were identified measuring the chromosome complement of each plate. The terminology of the chromosome morphology follows White (1973). Chromosome formulas were obtained according to Levan et al. (1964).

**Results**

**Morphological analysis**

The morphological analysis was carried out on the zinc violet from Cave del Predil, on the ssp. *subalpina* from Sauris di Sotto and on *V. calaminaria* from northwestern Europe. Exsiccata of *V. calaminaria* were included in this analysis in order to detect whether the two heavy metal-tolerant taxa show parallel variation in some morphological characters.

The results are as follows:

1) Shape of the leaves and stipules: no significative difference is evident among the three taxa.

2) Size of the flowers: it is almost impossible to estimate quantitative corolla characters in dried material. Therefore the comparison was only based on living plants. No significative difference seems to exist in the flower size between the zinc violet of Cave del Predil and that of Sauris di Sotto.

3) Length of the spur related to the calycine appendages: this character is reported in almost all taxonomical diagnoses; the ratio of these lengths seems to have a good taxonomical value. The length of the spur alone is very variable and cannot be considered as having diagnostic value. The results confirm the ratio value already known for the ssp. *subalpina:* the spur is about 2 times longer than the

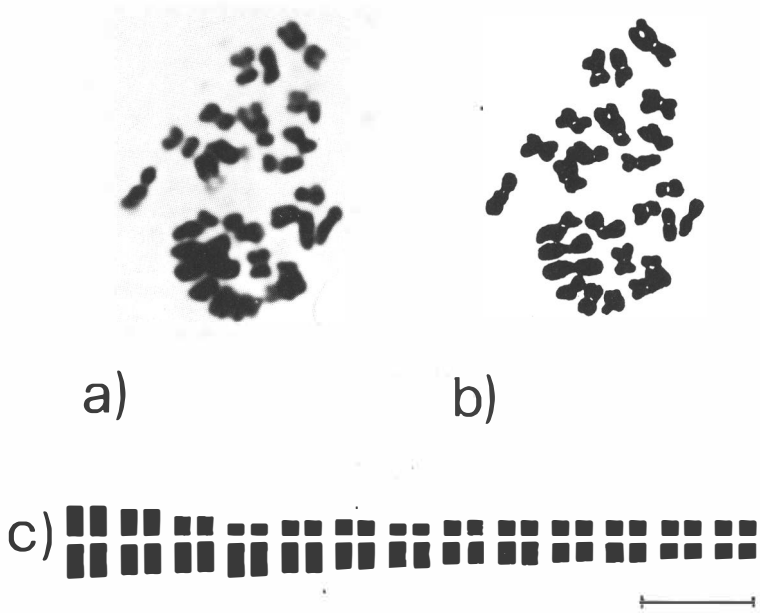


Fig. 1 - a) Somatic metaphase plate of *Viola tricolor* subsp. *subalpina,* (x 4750): b) tracing of a); c) caryotype: *2n = 26: 4M + 14m + 8 sm;* scale line 5 μm. (Expl. in text).

calycine appendages. The ratio value obtained for *V. calaminaria* and for the zinc violet from Cave del Predil is different: the spur is about 2-3 times longer than calycine appendages. The value 3 is the most frequent in the violet from Cave del Predil.

4) Length, width and their ratio of the seeds. The mean values are:

| | Length | Width | Length/Width |
|---|---|---|---|
| *Viola tricolor* subsp. *subalpina* | 1.49 ± 0.07 | 0.84 ± 0.11 | 1.77 |
| Zinc violet of Cave del Predil | 1.55 ± 0.09 | 0.77 ± 0.09 | 2.01 |
| *Viola calaminaria* | 1.87 ± 0.08 | 0.88 ± 0.06 | 2.12 |

The zinc violet from Cave del Predil has longer and narrower seeds than the closely related subsp. *subalpina,* with a ratio value close to that of the zinc violet from Belgium. Seed morphology has been never taken into account for taxonomic purposes in this species complex, but it seems to have some diagnostic value, as in this case.

**Caryological analysis**

Many chromosome counts on taxa of the *V. tricolor*-complex have been reported (Clausen, 1927; Tischler, 1934; Rohweder, 1937; Fothergill, 1941, 1944; Pettet,

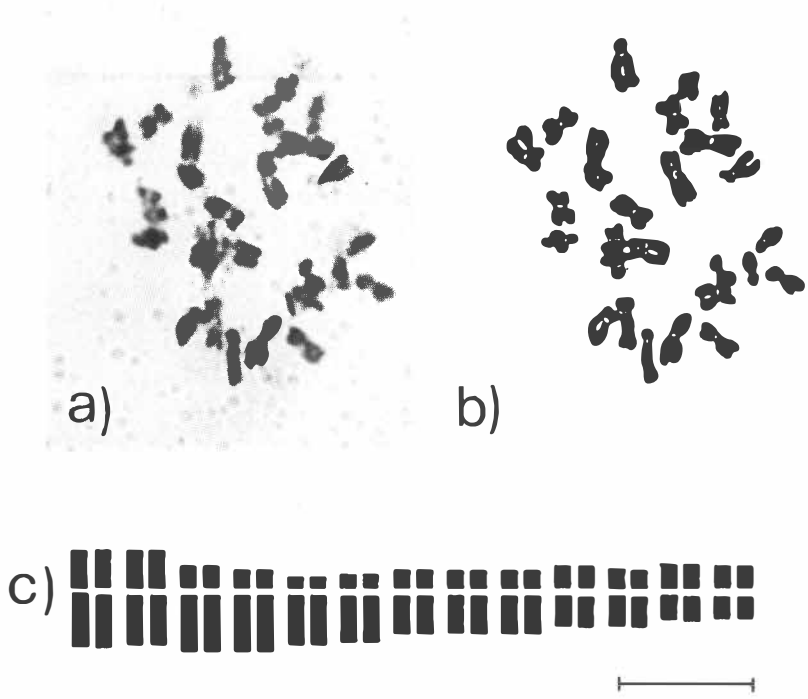


Fig. 2 - a) Somatic metaphase plate of *Viola tricolor* subsp. *subalpina* var. *raiblensis*, (x 3750); b) tracing of a); c) caryotype: *2n = 26 : 4M + 8m + 10sm + 4st;* scale line 5 μm. (Expl. in text).

1964, Lévêque & Gorenflot, 1969; Skalińska et al., 1971; Gadella, 1963). The basic number seems to be x = 13.

The zinc violets from eastern Belgium and of western Germany are tetraploids (Gadella, 1963; Ernst, 1965); they are probably derived from a 2n = 26 diploid ancestor (Heimans, 1961). On the basis of genecological investigations these two taxa appear to be autotetraploids and it seems that there is a single gene difference which is responsable for the blue and yellow phenotypes (Kakes & Everards, 1976).

The zinc violet from Cave del Predil and *V. tricolor* subsp. *subalpina* from Sauris di Sotto have the same chromosome number 2n = 26 (Figs 1a-b and 2a-b). Our count is new for populations of *V. tricolor* subsp. *subalpina* from the Alps, and is in accord with those carried out on populations of this taxon from Crna Gora (Yugoslavia) (Gadella & Kliphuis, 1972) and from Vysoké Tatry (Czechoslovakia) (Májovský et al., 1974).

The caryotype analysis in the wild population of *V. tricolor* subsp. *subalpina* from the meadows of Sauris di Sotto shows: two metacentric chromosome pairs with the centromere in the median point (M), seven metacentric pairs with the centromere in the median region (m) and four submetacentric pairs with the

centromere in the submedian region (sm) (Fig. 1c). According Levan et al. (1964) the chromosome formula is: *2n = 2x = 26: 4M + 14m + 8sm.* The chromosome length varies between 3 and 1.4 μm.

The caryotype of the zinc violet from the mine tailings of Cave del Predil is considerably different in chromosome morphology, compared to the wild population of subsp. *subalpina;* there are two metacentric chromosome pairs with the centromere in the median point (M), four metacentric pairs with the centromere in the median region (m), five submetacentric pairs with the centromere in the submedian region (sm) and two subtelocentric pairs with the centromere in the subterminal region (st) (Fig. 2c). Therefore the chromosome formula is: *2n = 2x = 26: 4M + 8m + 10sm + 4st.* The chromosome length varies between 4.30 and 2 μm.

**Discussion**

The results of the morphological analysis seem to confirm the observation made by Valentine et al. (1968) regarding the systematic position of *V. calaminaria* (DC.) Lej., which includes the violets growing on soils rich in zinc in Holland, Belgium and Germany. They have been provisionally grouped as subspecies within the *V. tricolor* - complex, but *V. calaminaria* may be probably best regarded as a variety of the very variable subsp. *subalpina* Gaudin. This observation seems to be based on the opinion of Heimans (1961), shared also by Gadella (1963), that on several grounds *V. calaminaria* could be derived from *V. tricolor* L. subsp. *alpestris* Ging apud DC (= *V. alpestris* (DC.) Wittr.). This latter taxon is considered as a synonym of *V. tricolor* L. subsp. *subalpina* Gaudin by Gams (1925). Many taxa have been described as varieties of subsp. *subalpina.* These varieties can be distinguished only by fairly subtle morphological differences, but it seems that also the ecological and phytosociological characters may be of importance; some varieties with alpic distribution show a clear ecological characterization; e.g.: var. *alpestris* Ging apud D C. seems to be restricted on talus slopes and xeric floodplains; var. *polychroma* (Kerner) Gams occurs in subalpine meadows (mostly Trisetum-meadows) and "Hochstaudenfluren"; var. *ramosa* (Gaudin) Gams grows in acidophytic xeric grasslands. At the moment more details on the distribution patterns and on the ecology of the various taxa are necessary to evaluate the importance of diacritic chorological and ecological characters.

From habit and texture, the zinc violet of Cave del Predil may be included without doubt in the subsp. *subalpina*-group. It is rather difficult to find good diagnostic characters separating the populations of Cave del Predil from those of Sauris di Sotto. The most striking character of immediate perception seems to be the ratio values length of the spur/length of the calycine appendages.

The comparison of the caryotypes (Figs 1c and 2c) gives the possibility to asses a genealogical relationships between these two taxa; most authors agree that matacentric chromosomes tend to be more primitive than asymmetrical chromosomes (Levitsky, 1931; Stebbins, 1971); the increase in asymmetry of chromosomes in the zinc violet complement supports the hypothesis that it may be evolved from plants of the *subalpina*-group.

As the relationships within the *subalpina*-group will be elucidated, it may be necessary to accord specific or subspecific rank to taxa with different chromosome complements, ecology, corology etc. For this reason, nomenclatural innovation would be premature at this time, and we regard the zinc violet of Cave del Predil as a variety of subsp. *subalpina.*

*Viola tricolor* L. subsp. *subalpina* Gaudin var. *raiblensis* Lausi et Cusma var. nova.

DIAGNOSIS: *Differt a taxis affinibus complexus Violae tricoloris subsp. subalpinae calcari appendicibus calycis 2 - 3plo longiore; seminibus longioris; chromosomatibus plerumque submetacentricis et subtelocentricis: 2n = 26.*

HABITAT: *Incolit sola ruderata glareosa zinco et plumbo divites metalli districtus "Cave del Predil".*

TYPUS: Cave del Predil (Udine), "Miniera di Raibl", alt. 920 m., 26.6.1981, legit Lausi (TSB).

The main difference between the two zinc violets, *V. calaminaria* and *Viola tricolor* subsp. *subalpina* var. *raiblensis,* is in the chromosomal complements. Morphological characters common to both taxa are the larger ratio spur/calycine appendages and the larger size of the seeds in respect to subsp. *subalpina.* These characters might be represent a convergent evolution as an adaptation to heavy metal soils, since they are present in chorologically widely separeted populations.

The var. *raiblensis* appears to have a high tolerance to toxic ions present in the soil; the uptake and accumulation of heavy metals in this zinc violet will be discussed in another paper.

**Concluding remark**

The intricate taxonomy of the *Viola tricolor*-complex may be related to the occurence of sympatric populations with genotypic, and hence phenotypic, differences which might have evolved as a response to different ecological conditions. We think that a more precise characterization of the ecology of different natural populations could be a fruitful starting point for caryological studies aiming at evaluating possible genetical differences among wild populations.

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