M. Erben

The significance of hybridization on the forming of species in the genus *Viola*

Abstract

Erben, M.: The significance of hybridization on the forming of species in the genus *Viola*. – Bocconea 5: 113-118. 1996. – ISSN 1120-4060.

Various evolutionary pathways involving hybridity are discussed, with examples taken from *Viola* sect. *Melanium*. Karyotype analysis will often enable reconstruction of parentage of hybridogenous polyploids. The study of hybrid populations shows that hybridity, including backcrosses, is a frequent phenomenon, and that mechanisms exist to balance karyotypes with uneven numbers of chromosomes, thus circumventing sterility. Karyotypes in this group have often evolved by (mostly descending) aneuploidy from a postulated original basis of $x = 11$ chromosomes, leading down to the level $x = 2$ (first reported here) which equals the lowermost number known so far in plants.

In the course of the last decades our knowledge on hybrids in vascular plants has increased rapidly. The importance of hybridization in evolution and speciation is discussed frequently, intensely and controversially in botanical literature. Some authors, e.g. Stebbins (1950), regard hybridization as a significant evolutionary factor, contrary to, e.g., Wagner (1970) who considers it as mere, insignificant evolutionary noise.

As nowadays no doubt remains on the role of hybridization in evolution, attention is increasingly focused on this phenomenon. Stace (1975) produced a catalogue of all known hybrids from the British Isles, listing 626 well substantiated cases of interspecific vascular plant hybrids, plus 122 possibly correctly interpreted ones. This is a surprising high number when compared to the about 3000 known species of the Flora of Britain. Presumably the British situation may be extrapolated to other regions of Europe as well.

If hybridization is such a common event, the following two questions become relevant: (1) Is hybridization an ephemeral phenomenon – a mistake in the production of species – or may it play a greater role in evolution? (2) How is the problem of hybrid sterility solved in different taxonomic groups?

I will use the genus *Viola* as example when discussing the mentioned problems. It is supposed to be a relatively old group due to its world-wide distribution and large number (c. 450) of species. A majority of the species occurs in the northern temperate zone. The genus has been divided into several sections, of which we shall only consider one: *V.* sect. *Melanium*. Its distribution is almost totally limited to Europe and W. Asia. This group has its centre of diversity on the hills and mountains of S. Europe, especially Italy and the Balkan Peninsula – my actual survey area.

The somatic chromosomes, especially in polyploids, are very small, so that their morphology is difficult to analyse exactly. Especially when examined in the summer season, some chromosomes tend to break in the region of the centromere, thus giving the illusion of a too high, and moreover fluctuating, chromosome number. The chromosomes themselves have a varied structure: besides metacentric chromosomes of various sizes, there are small sub-metacentric to sub-telocentric chromosomes. In no case were chromosomes with terminal centromeres observed. On the diploid level we may find the following chromosome numbers: $2n$ = 16, 18, 20, 22, 26, 28, and 34. A characteristic feature of some species are two long, conspicuous metacentric chromosomes, always present in plants with $2n$ = 16, 18, 26, and 28, whereas one group of species with $2n$ = 20 does have them but another group lacks them. The number in which this chromosome type occurs is of importance: in polyploid species, in particular, it permits to draw conclusions as to the nature and derivation of the constituent genomes. Thus species with $2n$ = 36 (e.g. *Viola epirota*) have four but others with $2n$ = 40 (e.g. *V. euboea*) only two large metacentric chromosomes. The occurrence of four metacentric chromosomes can be explained as resulting from simple doubling of the diploid number $2n$ = 18, either by autopolyploidy or by the cross of two diploid species with 2 metacentric chromosomes. For *V. euboea* with $2n$ = 40, on the other hand, one must postulate an allopolyploid origin, e.g. combining one 20-chromosome set with large metacentric chromosomes and one without such chromosomes.

It is more difficult to explain other polyploid chromosome numbers such as $2n$ = 48, 52, or even 96. The processes that must have taken place there are far more complex and probably went through several stages. To illustrate the significance of hybridization in species formation, some hybrid populations which I have studied may serve. As was to be expected, hybrids (including backcrosses) between two diploid species, *Viola elegantula* and *V. latisepala,* with the same chromosome number ($2n$ = 20) had the same number as the parental species, without any deviation or variation in number being observed (Fig. 1). The high fertility of these hybrids was remarkable – and can only result from undisturbed bivalent formation.

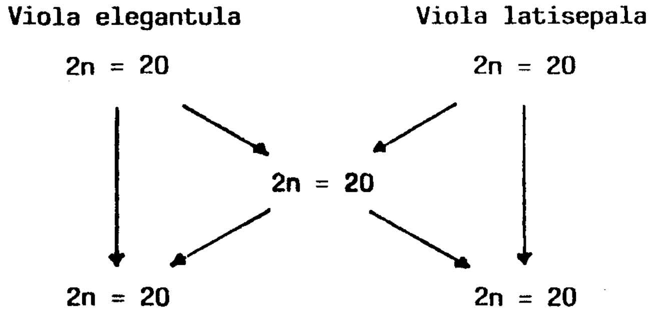

Fig. 1. A scheme of hybridization between *Viola elegantula* and *V. latisepala,* two diploid taxa with identical chromosome numbers.

The results are different for hybrids between species with different diploid chromosome numbers. In such a hybrid population, more than a single number may be found. The most frequent number obtained will correspond to the sum of the haploid chromosome numbers of the parents. For example, in the hybrid population *Viola velutina* ($2n$ = 18) × *Viola orphanidis* ($2n$ = 22) the primary number was $2n$ = 20 (consisting of one genome with 9 and a second with 11 chromosomes).

When individuals with an even chromosome number originate in this manner, they are as a rule fertile. Backcrossing with the parents will result in further strewing of the chromosome numbers. In our example, *Viola velutina* × *V. orphanidis*, the number $2n$ = 19 arises through backcrossing with *V. velutina*, and $2n$ = 21 through backcrossing with *V. orphanidis* (Fig. 2). Like any hybrids with an uneven number of chromosomes, these backcrosses are normally sterile.

The cytological situation of a hybrid population becomes even more complex and entangled when not only diploid species – as in our previous examples – but polyploids are involved. These hybrid populations, which are characterized by a marked aneuploid series of chromosome numbers, permit to gain insight into the structure of species in this section. The following example concerns a hybrid population found on Mt. Vasilitsa (Greece). Its polymorphic nature made it immediately clear that it was not composed exclusively of first generation hybrids. Three distinctive species, *Viola albanica*, *V. epirota* and *V. dukadjinica*, grow next to each other in that area and gave rise to an opulent hybrid population. *V. albanica* is a typical scree creeper with purple flowers and 34 chromosomes. *V. epirota* has a more tufted growth, blue-violet flowers, and 36 chromosomes. *V. dukadjinica* is a herbaceous species with yellow flowers and 52 chromosomes. The chromosome number expected for the F_1 generation of *V. epirota* × *V. dukadjinica*, $2n$ = 44 (18 + 26), was confirmed. Backcrossing of these hybrids with either parental species resulted in the numbers $2n$ = 40 (22 + 18) and $2n$ = 48 (22 + 26) [or upon reiteration of the backcross, $2n$ = 50 (24 + 26)], respectively. Backcrossing with *V. epirota* is rarely observed, probably due to a difference in flowering time. By analogy, the hybrid *V. albanica* × *V. dukadjinica* showed the number $2n$ = 43 (17 + 26), but also $2n$ = 44.

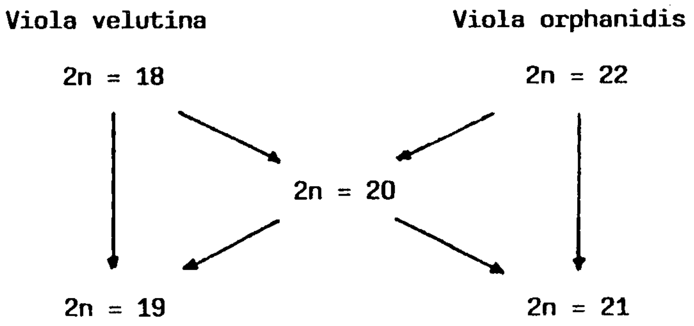

Fig. 2. A scheme of hybridization between *Viola velutina* and *V. orphanidis*, two diploid taxa with different chromosome numbers.

While the hybrids with 43 chromosomes were sterile, those with the stabilized number 2*n* = 44 showed regular seed production. Such up- or downward regulatory steps have been confirmed experimentally on several occasions in the genus *Viola*, e.g. by Clausen (1924); they may result through elimination of the single, non-homologous chromosome – or univalents – during the meiosis, or through its double splitting.

As for explaining the number 2*n* = 42, also observed, there are two possibilities (Fig. 3). (1) If the backcross of the hybrid *Viola epirota* × *V. dukadjinica* with *V. epirota* (2*n* = 40) hybridizes with the stabilized hybrid *Viola albanica* × *Viola dukadjinica* (2*n* = 44) the chromosome number 2*n* = 42 will result. (2) The result of reiterated backcrossing of the hybrid *V. epirota* × *V. dukadjinica* (2*n* = 50), if crossed with *V. albanica*, will again have 2*n* = 42 (25 + 17) chromosomes. One might argue that the number 2*n* = 42 could also have arisen by crossing among themselves the primary and secondary hybrids *V. epirota* × *V. dukadjinica* (2*n* = 44 and 2*n* = 40 chromosomes, respectively), without resorting to triple hybridity. The morphological characteristics of the 2*n* = 42 plant, however, clearly point to a participation of all three species.

My most important result is that hybrids with stabilized chromosome numbers are fertile, and that they are not rare. The observed facts, just discussed, contradict the view that hybrids of *Viola* sect. *Melanium* are formed each time anew, and due to their alleged sterility are of little importance for speciation. My studies show new ways in which the formation of given chromosome numbers can be explained.

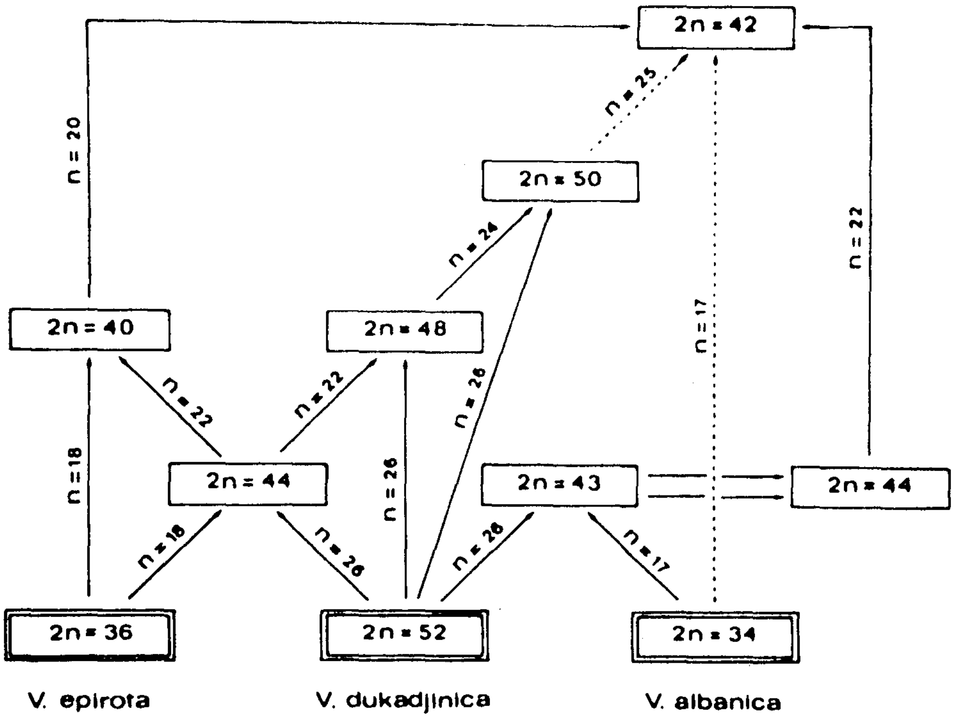

Fig. 3. Cytological analysis of a hybrid population and the three parent species, *Viola epirota*, *V. dukadjinica*, and *V. albanica*, on Mt Vasilitsa (Greece).

The number $2n = 34$ may serve as a good example. Küpfer (1971) tried to explain the formation of this number by the hybridization of species with $2n = 14$ and $2n = 20$ chromosomes, followed by a polyploidization step ($7 + 10 = 17 >> 34$), but two arguments contradict this hypothesis: (1) No suitable parent taxon with $x = 7$ could be found. (2) No such autopolyploidization could so far be demonstrated. A much more likely scenario, in such a case, would be stabilization of the $2n = 17$ hybrid by doubling of the unpaired chromosome, resulting in $2n = 18$.

By analogy to the processes just described for the Mt. Vasilitsa hybrid population, the following interpretation is possible. Through hybridization of two species with $2n = 20$ and $2n = 36$ chromosomes, respectively – two relatively common numbers – one gets a hybrid with $2n = 28$ ($18 + 10$). Through backcrossing twice with the $2n = 36$ parent, the number will increase to $2n = 34$ ($14 + 18 = 32 >> 16 + 18 = 34$).

In various species, the same individual numbers must certainly have originated several times. *Viola gracilis* from Ulu dağ in Turkey, for example, possesses a different karyotype than the same species with $2n = 34$ as it occurs in Greece. Furthermore, a distributional analysis of the various ploidy levels shows a remarkable concentration of diploid taxa in the northern part of Balkan Peninsula, whereas on the Greek mainland only polyploid taxa are to be found. The increase in ploidy level from north ($2x$) to south ($4x$-$10x$) curiously does not continue onto the islands: for example *V. cephalonica* has $2n = 20$ chromosomes.

In previous studies on the genus *Limonium* (Erben 1978) a similar increase of highly polyploid species ($4x$ to $6x$) in southern Greece and its off-shore islands was found. The assumption that the basal diploid species are to be looked for on the Greek mainland (Schmidt 1964) has not been confirmed.

As to the original basic number in *Viola* sect. *Melanium*, the present data support it to be $x = 11$. The taxa with $2n = 22$ chromosomes show several non-advanced characteristics. Through structural changes, e.g. the formation of the already-mentioned pair of large metacentric chromosomes, in a part of the species with 20 chromosomes, decreasing and increasing dysploidy has obviously taken place at an early stage (Fig. 4). The

$$x = 2 \leftarrow 3 \leftarrow 5 \leftarrow 6 \leftarrow 7 \leftarrow 8 \leftarrow 9 \leftarrow 10 \leftarrow \boxed{11} \rightarrow 12 \rightarrow 13 \rightarrow 17$$

Fig. 4. Descending and ascending aneuploidy in *Viola* sect. *Melanium*.

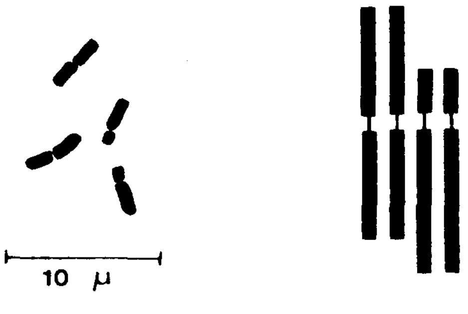

Fig. 5. Karyogram of *Viola modesta* s.l. from Turkey, with $2n = 4$

karyotype differentiation in this section is reflected by the formation of increasingly stronger asymmetrical idiogrammes, correlated with a decrease in basic chromosome number and transition to annual growth (Schmidt 1964). As an extreme, I found the chromosome number $2n = 4$ in an annual species of the *V. modesta* group from Turkey: this is the lowest chromosome number so far known for a vascular plant, and until now it had only been found in the family *Compositae,* e.g. in *Haplopappus* and *Antheropeas* (Fig. 5).

As we have seen, hybridization is an important factor in the formation of new species. In the genus *Viola,* hybrid sterility was bypassed through mechanisms to stabilize unbalanced genomes so as to permit normal propagation and thereby set the scene for the formation of new hybridogenous species. *V.* sect. *Melanium* shows a great polymorphism. It is built of a complex mixture of parental species, F_1 hybrids and backcrosses, forming a morphological and ecological continuum. The total amount of morphological variation far exceeds what is normally found in a group of intersterile species. From a taxonomic point of view, this section is considered 'critical' and 'very difficult', to the extent that a satisfactory classification of its weakly defined species is hardly possible with conventional methods of study. The only practicable solution is to reduce this reticulate 'hybrid complex' (in the sense of Grant 1976) to a few basic species. For this purpose, the knowledge of chromosome structure and experience gained through cultivating the plants are of great use.

References

Clausen, J. 1924: Increase of chromosome numbers in *Viola* experimentally induced by crossing. - Hereditas **5:** 29-32.

Erben, M. 1978: Die Gattung *Limonium* im südwestmediterranen Raum. - Mitt. Bot. Staatssamml. München **14:** 361-631.

Grant, V. 1976: Artbildung bei Pflanzen. - Berlin & Hamburg.

Küpfer, P. 1971: Contribution à l'etude cytologique et phylogénétique de la section *Melanium* Ging. du genre *Viola* L. - Compt. Rend. Hebd. Séances Acad. Sci., Sér. D, **272:** 1085-1088.

Schmidt, A. 1964: Cytotaxonomische Beiträge zu einer Neugliederung der Sektion *Melanium* der Gattung *Viola.* - Ber. Deutsch. Bot. Ges. **77:** 95-99.

Stace, A. 1975: Hybridization and the flora of the British Isles. - London.

Stebbins, L. 1950: Variation and evolution in plants. - New York.

Wagner, W. H. 1970: Biosystematics and evolutionary noise. - Taxon **19:** 146-151.

Address of the author:
Dr M. Erben, Institut für Systematische Botanik der Universität München,
Menzinger Straße 67, D-80638 München, Germany.