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Allozymic variation and relationships within *Viola* subsection *Viola* (Violaceae)

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Abstract. Allozyme markers from ten European taxa of *Viola* subsection *Viola* suggest that this group is allotetraploid, based on x = 5. All taxa had distinct multilocus phenotypes except *V. alba* subspp. *alba* and *scotophylla*, which were identical and different from subsp. *dehnhardtii*. Variation was consistently higher in Mediterranean populations than in North European ones. Hybridisation seems extensive but putative F_1 hybrids were distinctly less fertile than the parental species. Nevertheless, increased fertility in later-generation hybrids and shared band patterns among taxa indicate an important role of hybridisation and introgression in past and present evolution within the subsection. The octoploid *V. ambigua* shows affinity to *V. hirta* (tetraploid). The octoploid *V. suavis* probably originated from *V. pyrenaica* and other unidentified tetraploids, and high variability suggests polytopy or even polyphyly. The stoloniferous condition (series *Flagellatae*) seems to be primitive in the subsection but the reduction of stolons (series *Eflagellatae*) may have originated multiple times.

Key words: Violaceae, *Viola*, allozymes, introgression, reticulate evolution, hybrid speciation, paleopolyploidy.

European taxa of *Viola* L. subsection *Viola* (= *Uncinatae* Kupffer, *Scapigerae* W. Becker, *Curvato-pedunculatae* W. Becker) are intriguing in many respects, and their delimitation has been a topic of discussion throughout the last century (Becker 1903, 1910, 1925; Gerstlauer 1943; Muñoz Garmendia et al. 1993; Marcussen and Nordal 1998).

Subsection *Viola* is restricted to the temperate parts of Eurasia and North Africa and includes c. 20 species, depending on the delimitation of taxa (Table 1). *V. odorata* L. is the type of subsection *Viola* (Haesler 1982) and a diagnosis of the subsection, treated as a series, is given by Okamoto et al. (1993). Historically, considerable confusion has been expressed as to the taxonomic delimitations within subsection *Viola* (cf. among others Becker 1903, 1910; Schmidt 1961; Nordal 1996; Marcussen and Nordal 1998). Several factors may account for this. First, the subsection is morphologically quite distinct and homogeneous and taxa differ in relatively few characters. Second, widespread taxa usually show excessive morphological variation, both due to phenotypic plasticity (Bergdolt 1932) and regional differentiation (Marcussen et al. submitted). Third, taxonomic boundaries are often confused by interspecific hybridisation.

The species in the subsection are perennial and acaulescent, with lateral floriferous shoots modified to stolons, which may be reduced or

Table 1. Taxonomic delimitations within *Viola* subsection *Viola*. Chromosome number (if known), series, and geographic distribution is indicated for each taxon. Data are compiled from Becker (1910, 1918), Miyaji (1929), Melchior (1939), Yuzepchuk (1949), Valentine et al. (1968), Meusel (1978), Hultén and Fries (1986), Muñoz Garmendia et al. (1993), Okamoto et al. (1993), Anonymous (1998), Marcussen and Nordal (1998). The ambiguous taxa *V. indica* and *V. griffithiana* are not included here

Taxon	2n =	Series	Geographical distribution
V. ambigua Waldst. & Kit.	2n = 40	*Eflagellatae* Kitt.	SE Europe – Ural. Caucasus
V. chelmea Boiss. & Heldr.	2n = 20	*Eflagellatae*	Balkan (Croatia. Greece)
V. collina Besser	2n = 20	*Eflagellatae*	Scattered from C&N Europe – Lake Baikal. Kazakhstan (Tarbagatai) – Tadzikistan (Pamir). China, Korea & Japan
V. hirta L.	2n = 20	*Eflagellatae*	Europe & Caucasus – Lake Baikal. Kazakhstan (Tarbagatai). China (Tian Shan)
V. libanotica Boiss.	–	*Eflagellatae*	Lebanon (endemic)
V. pyrenaica Ramond	2n = 20	*Eflagellatae*	Pyrenees. Alps. Appeninnes. Balkan. Caucasus. Atlas
V. sandrasea Melch.	–	*Eflagellatae*	Turkey: Mugla (endemic). – Possibly extinct; known from the type collection only
V. thomasiana Perr. & Song.	2n = 20	*Eflagellatae*	Alps (endemic)
V. alba Besser ssp. *alba* (= *V. virescens* Jord.)	2n = 20	*Flagellatae* Kitt.	N & W of the Alps, scattered to Caucasus. – Naturalised in Sweden: Öland
V. alba ssp. *dehnhardtii* (Ten.) W. Becker	2n = 20	*Flagellatae*	W & C Mediterranean region
V. alba ssp. *scotophylla* (Jord.) Nyman	2n = 20	*Flagellatae*	SE Europe – Caucasus. Scattered W of the Alps
V. alba ssp. *sintenisii* (W. Becker) W. Becker	–	*Flagellatae*	N Iran (Elburs) – S Turkmenistan (Kopet Dagh). – Naturalised in Kazakhstan & Tadzikistan
V. canescens Wall.	–	*Flagellatae*	Himalaya – S China
V. cretica Boiss. & Heldr.	2n = 20	*Flagellatae*	Greece: Crete (endemic)
V. curvicalcarata W. Becker & Boiss.	–	*Flagellatae*	China
V. hondoensis W. Becker & Boiss.	2n = 20	*Flagellatae*	Japan
V. ignobilis Rupr.	–	*Flagellatae*	Caucasus. Romania
V. jaubertiana Marès & Vigin.	2n = 20	*Flagellatae*	Spain: Majorca (endemic)
V. odorata L.	2n = 20	*Flagellatae*	C Europe, Mediterranean region, Caucasus. Macaronesia Kazakhstan – Tadzikistan. India? – Naturalised throughout the temperate zone
V. pilosa Blume (= *V. serpens* Wall.)	2n = 20	*Flagellatae*	Afghanistan – Himalaya – S China, Sri Lanka & Malesia
V. principis Boiss.	–	*Flagellatae*	S China
V. suavis M.Bieb.	2n = 40	*Flagellatae*	C&S Europe – S Turkmenistan (Kopet Dagh). N Africa (Atlas) Kirgyizistan – Tadzikistan. Cashmere? – Naturalised in N Europe and W Iberia
V. yunnanensis W. Becker & Boiss.	–	*Flagellatae*	China (Sichuan, Yunnan, Hainan). Korea

absent (Okamoto et al. 1993). Chasmogamous, entomophilous flowers are produced in early spring, and cleistogamous flowers are produced during favourable periods throughout the rest of the growth season (Redbo-Torstensson and Berg 1995). Characteristics of the subsection are the globose, non-ballistic capsules borne on decumbent pedicels at maturity. The seeds have conspicuous elaiosomes and are dispersed by ants (Culver and Beattie 1980). Most species belong to the deciduous forest element, growing in glades or scrubs, or in permanently open areas such as steppes or mountains. The habitats often have an element of disturbance, and at least some of the European species seem to be favoured by human activity (Beattie 1974, Grime et al. 1990, Kosonen et al. 1996, Marcussen and Nordal 1998).

The chromosome number 2n = 20 is most common among the investigated species in subsection *Viola*, but *V. ambigua* and *V. suavis* are reported to have 2n = 40 (Miyaji 1929; Clausen 1929; Schmidt 1961, 1964; Muñoz Garmendia et al. 1993; Okamoto et al. 1993; Marcussen and Nordal 1998).

Subsection *Viola* has traditionally been divided into two series, *Flagellatae* and *Eflagellatae*, based on whether stolons are present or not (Table 1; Becker 1925, Melchior 1939, Schmidt 1964, Okamoto et al. 1993). Twelve species are included in series *Flagellatae* (Table 1). Six species have a mainly European-Mediterranean distribution, i.e. *V. alba*, *V. cretica*, *V. ignobilis*, *V. jaubertiana*, *V. odorata*, and *V. suavis*. Morphologically similar is the Japanese *V. hondoensis*. Recently, Okamoto et al. (1993) included five more species from South and East Asia, i.e. *V. canescens*, *V. curvicalcarata*, *V. pilosa*, *V. principis*, and *V. yunnanensis*. These species were previously included in subsection *Serpentes* W. Becker (but see de Candolle 1824).

Series *Eflagellatae* includes eight species (Table 1). Four are temperate, mainly with European-Central Asian distributions, i.e. *V. ambigua*, *V. collina*, *V. hirta*, and *V. thomasiana*. The remaining four species, *V. chelmea*, *V. libanotica*, *V. pyrenaica*, and *V. sandrasea*, are all relictual with narrow endemic or disjunct distributions, and native to the high montane and alpine regions of Central Europe, North Africa, and the Middle East (Melchior 1939, Schmidt 1964).

The *Viola alba* complex consists of several more or less vicarious races, treated as subspecies of *V. alba* or as separate species (Becker 1910, 1918; Yuzepchuk 1949; Schmidt 1961; Valentine et al. 1968; Hess et al. 1970). The subspecies *alba* and *scotophylla* are largely sympatric in Central Europe but subsp. *alba* is more common north and west of the Alps, whereas subsp. *scotophylla* is more common from the Alps and eastwards to the Caucasus. Subsp. *dehnhardtii* is Mediterranean and parapatric to both subspp. *alba* and *scotophylla*, and a distinct transition zone is found across southern Europe from northern Spain to Greece (Becker 1910, Strid 1986, Muñoz Garmendia et al. 1993). Subsp. *sintenisii* and *V. cretica* are endemic to the mountains of Asia Minor (Elburs to Kopet Dagh) and Crete, respectively (Becker 1910, 1918; Yuzepchuk 1949; Schmidt 1961).

Viola jaubertiana is a narrow endemic, restricted to Majorca. Becker (1910) included it in *V. alba* but later authors recognise it as a separate and well-defined species, possibly representing an isolated, relictual lineage within the subsection (Tchourina 1909, Chodat 1924, Schmidt 1961).

Viola odorata is now widely distributed due to cultivation outside its original range in southern Europe and the Mediterranean region (Hultén and Fries 1986, Nordal 1996). Becker (1910) described it as morphologically relatively invariable (but see Marcussen et al. submitted). *V. ignobilis*, a subalpine, minute species native to Romania and the Caucasian mountains (Becker 1918, Yuzepchuk 1949, Grintescu et al. 1955), and *V. hondoensis*, a species endemic to Japan (Becker 1908, 1918), are morphologically reminescent of *V. odorata*.

Viola suavis, like *V. odorata*, is locally naturalised in central and northern Europe, partly due to cultivation (Gams 1925, Munõz Garmendia et al. 1993, Marcussen and Nordal

1998). It is a critical taxon due to extensive morphological variability, and much confusion has been expressed as to its internal substructure and its delimitation from related taxa, e.g. *V. alba*, *V. jaubertiana*, and *V. odorata* (see Marcussen and Nordal 1998 for survey and discussion).

Viola ambigua is a steppe plant reaching westwards to eastern Europe. There has been some confusion as to its delimitation from *V. thomasiana* and similar forms of *V. hirta* (Schmidt 1961), hence the name.

Viola collina, in spite of its wide distribution (most of the temperate parts of Eurasia), is morphologically rather uniform (Becker 1910, 1918; Marcussen et al. submitted). Related is probably *V. thomasiana* (Gerstlauer 1943), an endemic to the Alpine coniferous forests. Both species are more strongly connected to boreal forests than other taxa in the subsection.

Viola hirta is common throughout the central parts of Europe and the nemoral vegetation zone. It is a morphologically variable species, but little of its variation appears to be geographically structured (Becker 1910, Schmidt 1961; Marcussen et al. submitted).

Viola pyrenaica is a highly disjunct, alpine species. Some morphological variation has been reported among its regions of distribution but it is unclear whether this deserves taxonomic recognition (Becker 1918, Yuzepchuk 1949).

There are only weak barriers against interspecific hybridisation in subsection *Viola*, like in the genus in general, and hybridisation frequently takes place wherever species meet (Bethke 1882; Erdner 1907; Becker 1910; Schnarf 1922; Gerstlauer 1943; Schöfer 1954; Schmidt 1961; Dizerbo 1967, 1968; Valentine 1975; Hiemeyer 1992). Figure 1 shows all reports of spontaneous hybrids between taxa of the subsection in Europe. Hybrids are usually somewhat fertile (with up to 5% pollen fertility), even some hybrids between taxa at different ploidal levels, i.e. *V. ambigua* × *hirta* and *V. odorata* × *suavis* (Erdner 1907, Schmidt 1961). So far, hybrid swarms have been identified only between *V. hirta* and the three taxa *V. alba*, *V. collina*, and *V. odorata* (Fig. 1; Schöfer 1954, Schmidt 1961). In these hybrid combinations, later-generation offspring shows increased chromosome numbers and increased fertility, and a recombination of parental morphological characters.

Occasionally, species in subsection *Viola* also form spontaneous hybrids with species in the caulescent subsection *Rostratae* Kupffer. These hybrids are vigorous but completely sterile (Fig. 1; Becker 1910, Clausen 1929, Dodd and Gershoy 1943, Gershoy 1934).

In spite of evident macro-morphological differentiation, subsections *Viola* and *Rostratae* are shown to be phylogenetically closely related (Miyaji 1929, Clausen 1929, Ballard et al. 1999). Besides the retained ability to interbreed, the two subsections show strong similarities in style anatomy (Clausen 1929), and they share the chromosome number $n = 10$. This number appears to be basic in section *Viola* sensu Ballard et al. (1999) whereas the basic number for the genus as a whole, as well as for large parts of the family, is $x = 6$ (Clausen 1929, Miyaji 1929).

Compared with subsection *Rostratae*, subsection *Viola* shows a derived state in several morphological characters, e.g. general growth form (acaulescence), lateral shoots (bibracteolate stolons or absence thereof), capsules (non-ballistic), and seeds (large with conspicuous elaiosomes) (Clausen 1929, Valentine 1962, Beattie 1974, Okamoto et al. 1993). In a phylogenetic study of the genus based on ITS sequences, Ballard et al. (1999) showed that subsection *Rostratae* consists of several independent, early-diverging lineages within section *Viola*. Thus, this subsection appears to be paraphyletic and, apparently, ancestral to numerous derived groups with more limited distributions, among which subsection *Viola* is the only group restricted to the Old World.

Considerable re-structuring and new delimitations of infrageneric groups resulted from this phylogenetic analysis, and the taxonomy and preliminary nomenclature of infrageneric groups referred here therefore follow Ballard et al. (1999).

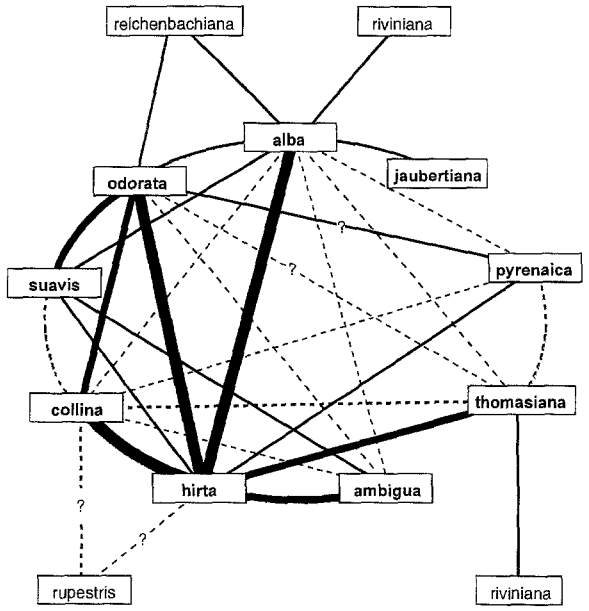


Fig. 1. Interspecific hybridisation in *Viola* subsection *Viola*. Their rare natural hybrids with members of subsection *Rostratae* Kupffer are also shown, i.e. with *V. reichenbachiana* Jord., *V. riviniana* Rchb., and possibly *V. rupestris* F. W. Schmidt. Broken lines indicate hybrids with unknown fertility; thin lines indicate sterile or mostly infertile hybrids; intermediate lines indicate fertile hybrids (up to ca. 5%); thick lines indicate fertile hybrids were introgression with either parent has been reported. Doubtful records are labelled with question marks. Based on data from Erdner (1907), Becker (1910), Schnarf (1922), Gerstlauer (1943), Schöfer (1954), Schmidt (1961), Dizerbo (1967, 1968), Valentine (1975), and Hiemeyer (1992)

The aim of this study is to elucidate relationships among European taxa of subsection *Viola* by means of allozyme markers, in order to obtain a fuller understanding of the evolutionary pathways in this critical group. There is evidence that reticulate evolution is important in subsection *Viola*, and allozymes have proven useful in tracing reticulate evolutionary patterns, both at homoploidal and polyploidal levels.

Materials and methods

Materials. A total of 556 plants from 76 European populations was included in the analyses (Table 2). Allozyme markers were scored for 553 individuals of *V. alba* (subspp. *alba*, *scotophylla*, and *dehnhardtii*), *V. ambigua*, *V. collina*, *V. hirta*, *V. jaubertiana*, *V. odorata*, *V. pyrenaica*, and *V. suavis*, and several generations of the five putative hybrid combinations *V. alba* × *hirta*, *V. alba* × *odorata*, *V. collina* × *hirta*, *V. collina* × *odorata*, and *V. hirta* × *odorata*. A subset of 241 plants was cultivated in a greenhouse, providing morphology and fertility data. The cultivated plants were either raised from seeds received from European botanical gardens (s), or collected from native European populations by L. Borgen (LB), B. Jonsell (BJ), T. Marcussen (TM), I. Nordal (IN), or M. Ursin (MU; Table 2). Unfortunately, three vigourous putative F_2 hybrid individuals (one from population 3 and two from population 53) died due to mismanagement before allozymes were scored and were therefore included in the fertility and morphology analyses only. All putative hybrids collected in the field with full band additivity compared to the parental taxa were interpreted and treated as F_1 hybrids. Hybrids with incomplete band additivity were collectively denoted F_n hybrids, and their offspring F_{n+1}. Plants raised from cleistogamous putative F_1 hybrid seeds were denoted F_2, and the next generation F_3.

Methods. Tissue preparation and electrophoretic procedures generally followed Morden et al. (1987). Six enzyme systems were investigated, i.e. AAT (aspartate aminotransferase), AMP (aminopeptidase), GPI (glucose-6-phosphate isomerase), IDH (NADP-isocitrate dehydrogenase), PGM (phosphoglucomutase), and SKD (shikimate dehydrogenase). A modification of Wendel and Weeden's (1989) buffer-system 1 (the "D" system: histidine-citrate, pH 6.5) was used for IDH, PGM, and SKD, and a modification of buffer-system 6 (the "Ashton" system: Li-borate/tris-citrate, pH 8.1) for AAT, AMP, and GPI. Problems related to slime production in *Viola* (resulting in viscous homogenates, smeary gels etc.) were largely avoided by using only young, folded leaves.

Because it is difficult to assign alleles to specific loci without analysing segregation of progeny (Werth 1989, Kephart 1990), allelic bands were labelled alphabetically from the most anodal band. Allelic bands (heterodimers excluded) were scored as present or absent and analysed using UPGMA (unweighted-pair groups method analysis; Sokal and Michener 1958) with squared Euclidean distance and PCO (principal coordinate analysis; Gower 1966) with Dice's distance.

Table 2. Analysed materials of *Viola*. Number of individuals per taxon and locality is indicated. Materials were collected by L. Borgen (LB), B. Jonsell (BJ), T. Marcussen (TM), I. Nordal (IN), M. Ursin (MU), or grown from seeds (s) received from European botanical gardens (B.G.). All hybrids with full additivity of parental enzyme bands are interpreted as F_1. Abbreviations: *Fr* France, *Ge* Germany, *Hu* Hungary, *It* Italy, *Li* Lithuania, *No* Norway, *Po* Poland, *Se* Sweden, *Sp* Spain, *Sw* Switzerland

Locality and population number	Collectors	*alba* subsp. *alba*	*alba* subsp. *scotophylla*	*alba* subsp. *dehnhardtii*	*ambigua*	*collina*	*hirta*	*jaubertiana*	*odorata*	*pyrenaica*	*suavis*	*alba* × *hirta* F_1	*alba* × *hirta* F_2	*alba* × *odorata* F_1	*alba* × *odorata* F_n	*collina* × *hirta* F_1	*collina* × *hirta* F_2	*collina* × *hirta* F_3	*collina* × *hirta* F_n	*collina* × *hirta* F_{n+1}	*collina* × *odorata* F_1	*collina* × *odorata* F_2	*hirta* × *odorata* F_1	*hirta* × *odorata* F_2	Sum individuals
1 Fr Ain: Bouis	TM	3	6	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	11
2 Fr Ain: Bourg-en-Bresse, Rue d'Ypres	TM	7	.	.	.	.	.	.	6	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	14
3 Fr Ain: btw. Veronnat & Reinat	TM	3	.	.	.	.	.	.	.	.	.	2	3	.	.	.	.	.	.	.	.	.	.	.	8
4 Fr Ain: Ceyzeriat, Mt. July	TM	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
5 Fr Ain: Les Conches	TM	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
6 Fr Ain: Meillonas	TM	5		.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
7 Fr Alpes-Maritimes: btw. Le Bar & Gourdon	TM	.	.	8	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	10
8 Fr Alpes-Maritimes: Les Barres, nr. La-Colle-sur-Loup	TM	.	.	2	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	4
9 Fr Alpes-Maritimes: Parc St Donat, nr. La-Colle-sur-Loup	TM	.	.	4	.	.	.	.	5	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	13
10 Fr Alpes-Maritimes: Plateau de Caussols	TM	.	.	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	3
11 Fr Alpes-Maritimes: St Jeannet 1	IN	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2
12 Fr Alpes-Maritimes: St Jeannet 2	IN	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8
13 Fr Alpes-Maritimes: St Jeannet 3	TM	.	.	.	.	.	.	.	.	.	7	.	.	.	.	.	.	.	.	.	.	.	.	.	7

14	Fr Bouches-du-Rhône: Cassis, V^{on} d'En-Vau	TM	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
15	Fr Bouches-du-Rhône: Géménos, St Pons	TM	.	.	4	.	.	.	.	1	.	.	.	.	6	3	.	.	.	.	.	.	.	.	.	.	.	.	14
16	Fr Corsica: Bocca di Bonassa, nr. Bonifato	IN	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
17	Fr Corsica: Bridge of Figarella, nr. Calenzana	IN	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
18	Fr Corsica: nr. Calenzana	IN	.	.	2	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
19	Fr Haute-Savoie: Talloires, nr. Annecy	TM	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
20	Fr Isère: btw. Allemont & Le-Rivier-d'Allemont, 1000 m alt.	TM	.	.	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	10		
21	Fr Isère: btw. Col Luitel & Séchilienne, 700 m alt.	TM	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
22	Fr Isère: btw. Uriage-les-Bains & Chamrousse, 900 m alt.	TM	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
23	Fr Jura: La Saugiat, nr. La Marre	TM	.	.	.	.	.	.	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	9		
24	Fr Loire: Renaison, nr. Roanne	TM	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
25	Fr Var: Esterel, Gorges Blavet	TM	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
26	Fr Var: St-Cyr-sur-Mer, Les Lecques	TM	.	.	.	.	.	.	.	1	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7
27	Fr Yvelines: Le Chaisnay, nr. Versailles	TM	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
28	Fr Yvelines: St-Germain-en-Laye	TM	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1

Table 2. (Continued)

Locality and population number	Collectors	*alba* subsp. *alba*	*alba* subsp. *scotophylla*	*alba* subsp. *dehnhardtii*	*ambigua*	*collina*	*hirta*	*jaubertiana*	*odorata*	*pyrenaica*	*suavis*	*alba* × *hirta* F_1	*alba* × *hirta* F_2	*alba* × *odorata* F_1	*alba* × *odorata* F_n	*collina* × *hirta* F_1	*collina* × *hirta* F_2	*collina* × *hirta* F_3	*collina* × *hirta* F_n	*collina* × *hirta* F_{n+1}	*collina* × *odorata* F_1	*collina* × *odorata* F_2	*hirta* × *odorata* F_1	*hirta* × *odorata* F_2	Sum individuals
29 Ge Hessen: Frankfurt a.M. Botanical Garden	s	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
30 Ge Niedersachsen: Göttingen (Bot. Gard.)	s	.	.	.	.	.	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9
31 Ge Niedersachsen: Harz, Hörne (Bot. Gard.)	s	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
32 Hu Pest: Gödöllö Botanical Garden	s	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
33 Hu Pest: Szarvasko (Bot. Gard.)	s	.	.	.	.	.	.	.	.	.	7	.	.	.	.	.	.	.	.	.	.	.	.	.	7
34 It Sicily: Floresta	TM	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1
35 It Sicily: nr. Linguaglossa	TM	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
36 It Veneto: Genoa Botanical Garden	s	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	2
37 Li Vilnius: Vilnius	IN	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
38 No Akershus: Blakstad	MU	.	.		.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
39 No Buskerud: Hole, Bønsnes	TM	.	.	.	.	4	6	.	.	.	.	.	.	.	.	9	.	.	.	.	.	.		.	19
40 No Buskerud: Hole, Leine	TM	.	.	.	.	2	17	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	22
41 No Buskerud: Hole, Leinestranda	TM	.	.	.	.	.	5	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	9
42 No Buskerud: Hole, Nøstret	TM	.	.	.	.	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9
43 No Buskerud: Hole, Svarstad	TM	.	.	.	.	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
44 No Buskerud: Hurum, Mølen inlet	TM,IN	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8

45	No Buskerud: Hurum, Ranvikholmen inlet	IN	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
46	No Buskerud: Hurum, Toftcholmen inlet	TM,IN	.	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8
47	No Buskerud: Modum, nr. Vikersundbakken	TM	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
48	No Buskerud: Ringerike, btw. Burudåsen & Åsa	TM	.	.	.	.	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8
49	No Oppland: Lunner, Roa	MU	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
50	No Oslo: Blindern (cultivated)	TM	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
51	No Oslo: btw. Slemdal & Kringsjå	MU	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
52	No Oslo: Bygdøy, nr. Rodeløkka Café	MU	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
53	No Oslo: Ekebergskråninga, Nedre Bekkelaget	TM,MU	.	.	.	.	9	.	.	6	.	.	.	.	.	.	.	.	.	.	.	.	8	2	.	.	25
54	No Oslo: Ekebergskråninga, Sjømannsskolen	TM	.	.	.	.	.	.	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
55	No Oslo: Ekebergskråninga, Øvre Bekkelaget	TM	.	.	.	.	15	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	18
56	No Oslo: Hovedøya island NE	TM	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
57	No Oslo: Hovedøya island S	TM	.	.	.	.	1	8	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	10
58	No Oslo: Hovedøya island SW	TM	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
59	No Oslo: Jar, Jarfossen	MU	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
60	No Oslo:Lindøya island	TM,IN	.	.	.	.	7	8	.	2	.	.	.	.	.	.	11	4	3	.	.	4	2	2	3	46
61	No Oslo: Nordstrand, Breidablikkveien	TM	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
62	No Oslo: Nordstrand, Skansebakken	TM	.	.	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
63	No Oslo: Skøyen, Silkestrå	TM,IN	.	.	.	.	6	5	.	1	.	.	.	.	.	.	1	.	.	.	.	1	.	8	.	22

Table 2. (Continued)

Locality and population number	Collectors	*alba* subsp. *alba*	*alba* subsp. *scotophylla*	*alba* subsp. *dehnhardtii*	*ambigua*	*collina*	*hirta*	*jaubertiana*	*odorata*	*pyrenaica*	*suavis*	*alba* × *hirta* F_1	*alba* × *hirta* F_2	*alba* × *odorata* F_1	*alba* × *odorata* F_n	*collina* × *hirta* F_1	*collina* × *hirta* F_2	*collina* × *hirta* F_3	*collina* × *hirta* F_n	*collina* × *hirta* F_{n+1}	*collina* × *odorata* F_1	*collina* × *odorata* F_2	*hirta* × *odorata* F_1	*hirta* × *odorata* F_2	Sum individuals
64 No Oslo: Tøyen, nr. railway subpass	TM	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
65 No Oslo: Tøyen, Ola Narr	TM	.	.	.	.	4	7	.	.	.	.	.	.	.	.	5	.	.	.	.	.	.	.	.	16
66 No Telemark: Kragerø, Valberg	TM	.	.	.	.	.	13	.	5	.	25	.	.	.	.	.	.	.	.	.	.	.	.	.	43
67 No. Østfold: Jeløya, Alby (Galleri F15)	TM	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
68 No Østfold: Jeløya, Fuglevik	TM	.	.	.	.	.	13	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	12	2	32
69 Po Poznan: Poznan botanical garden	s	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	10	.	.	.	.	13
70 Se Öland: Borgholm	LB, BJ	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	2
71 Se Öland: Räpplinge, N Borgholm alvar	IN,BJ	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
72 Se Öland: Sandby, Ekelunda alvar	IN,BJ	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
73 Se Öland: Vickleby	IN,BJ	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
74 Sp Majorca: Sa Fosca (Bot. Gard.)	s	.	.	.	.	.	.	11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	11
75 Sp Tenerife: Los Portelas	TM	.	.	.	.	.	.	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
76 Sw St Gallen: St Gallen Botanical Garden	s	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Number of individuals:		20	9	37	3	79	134	11	80	1	61	2	3	8	3	34	4	3	3	10	14	4	26	7	556

To estimate the degree of stolon production, maximal internode length on lateral shoots was scored. Ripe cleistogamous capsules were collected for fertility estimation (seeds per capsule). Seed germination was obtained under long-day conditions at 15/10 °C after nine weeks of out-door stratification at −5 to 9 °C under short-day conditions.

Results

Variation within taxa. The banding patterns seen on the gels were in accordance with enzyme subunit composition known from previously studied vascular plants: AMP, PGM, and SKD were monomeric, and AAT, GPI, and IDH dimeric (Kephart 1990). However, duplication of putative loci within subcellular compartments was observed for all six enzyme systems (cf. Culley and Wolfe 2000), often appearing in distinct regions of the gel in the 2n = 20 taxa. Further duplications were observed in the 2n = 40 species, *V. ambigua* and *V. suavis*, and allozyme bands in the 2n = 40 taxa corresponding to a single putative locus in the 2n = 20 taxa will be referred to as one locus below.

A total of 58 allozyme markers in 16 putative loci was scored. Their migration relative to the front (R_f values) is shown in Fig. 2, and putative loci are indicated. The most anodal loci of AAT and GPI, *Aat-1* and *Gpi-1*, were not interpretable. Two putative loci were interpreted in AAT, GPI, and SKD, three in AMP and PGM, and four in IDH. *Idh-1/2* and *Idh-3/4* were expressed in separate subcellular compartments. The slower PGM region was interpreted as consisting of two

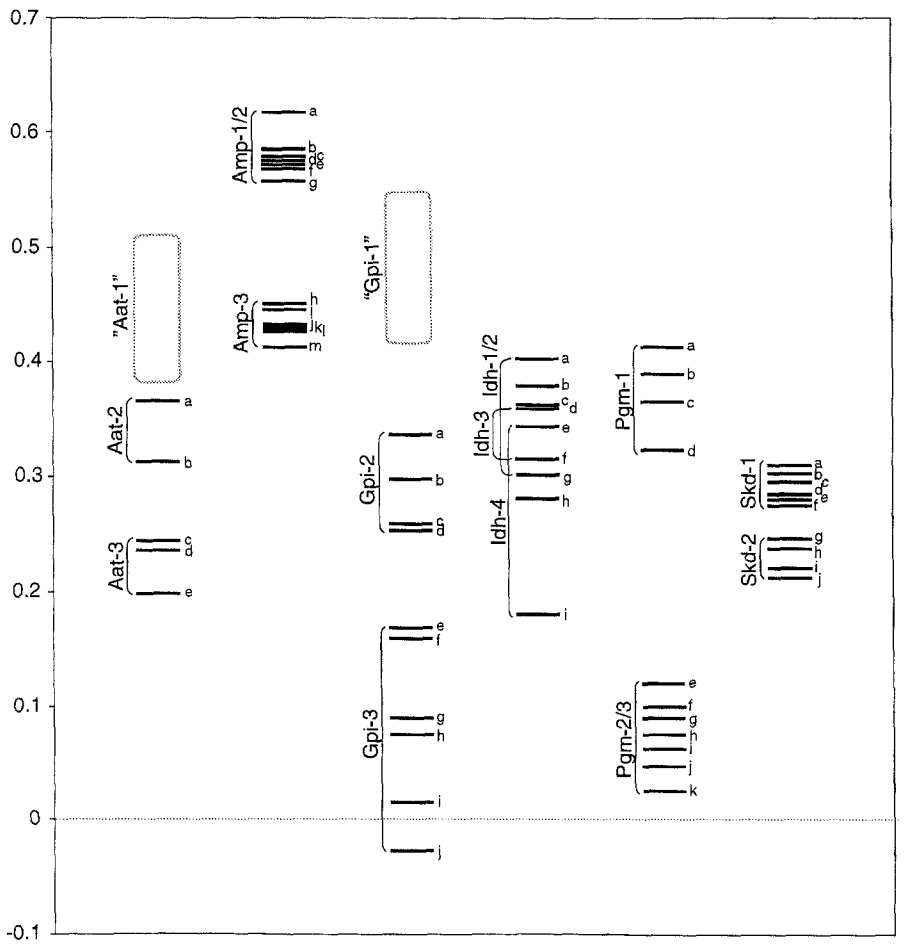


Fig. 2. Allelic migration relative to the migration front (R_f values) in *Viola* subsection *Viola*. A total of 58 putative alleles and 16 loci was observed in the six enzyme systems AAT, AMP, GPI, IDH, PGM, and SKD. Regions with uninterpretable activity are indicated with open boxes (i.e. *Aat-1* and *Gpi-1*)

duplicate loci, *Pgm-2* and *Pgm-3*, between which allelic bands were shared.

The largest proportion of allelic bands (20 out of 58, or 37%) were unique to single taxa (subsp. *scotophylla* included in subsp. *alba*; see below) but only nine were actually fixed and diagnostic to taxa. Allelic bands shared by many taxa were remarkably rarer than bands shared by few taxa and only two alleles were shared by all nine taxa (*Idh-h* and *Pgm-h*).

A total of 61 multilocus phenotypes was found in the ten taxa, as listed in Table 3 with reference to taxa and populations. Distinct enzyme phenotypes were revealed for all taxa except *Viola alba* subsp. *alba* and subsp. *scotophylla*. While populations in northern Europe, including Scandinavia, were mostly monomorphic, the levels of variation increased markedly southwards to the Mediterranean region, where occasionally much intrapopulation variation was found. Some variation, both within and among taxa, was due to apparent lack of expression at particular loci, i.e. *Aat-2* (occasionally in *V. alba* subsp. *dehnhardtii*), *Aat-3* (exceptionally in *V. hirta*, *V. odorata*, and *V. suavis*), *Amp-1* (*V. jaubertiana* and *V. pyrenaica*, also occasionally in *V. alba* subsp. *dehnhardtii*), *Amp-2* (*V. alba* subsp. *alba*, subsp. *scotophylla*, prevailing in *V. collina*, and exceptionally in *V. hirta*), *Pgm-2* (exceptionally in *V. alba* subsp. *dehnhardtii* and *V. suavis*), and *Skd-1* (*V. jaubertiana*, occasionally in *V. hirta*).

No variation was found within the single populations of *Viola. ambigua*, *V. jaubertiana*, and *V. pyrenaica*.

Viola alba was variable, and included alone 23 of the 61 observed multilocus phenotypes. The variation showed a strong geographical structure. In most cases, subsp. *alba* (three phenotypes) and subsp. *scotophylla* (two phenotypes) were enzymatically indistinguishable but both had rare, slightly deviating phenotypes approaching subsp. *dehnhardtii* in allelic composition (*Amp-i*, *Gpi-f*, *Skd-i*). On the other hand, subsp. *dehnhardtii*, with 19 multilocus phenotypes, was distinct and far more variable than the other two subspecies, differing from subspp. *alba*/*scotophylla* in AAT (*e* vs. *d*), AMP (*bd* vs. *d*), and IDH (*c* vs. *f*). Differences were usually found for GPI and SKD as well, i.e. *Gpi-g* was fixed in subsp. *dehnhardtii* but rare in subsp. *alba*, and *Skd-d* was fixed in subspp. *alba*/*scotophylla* but rare in subsp. *dehnhardtii*. Corresponding allelic variation was detected in AMP (*i* or *k*) and SKD (*g* or *j*). Additional variation in subsp. *dehnhardtii* was observed in AAT (*be* or *e*), AMP (*bd*, *b*, or *bf*), GPI (*b* or *c*), IDH (*h*, *he*, or *e*), PGM (*hi* or *i*), and in SKD (*bg*, *dg*, *bdg*, or *di*). It is noteworthy that the rare *Amp-f* allele was shared only with *V. odorata*. The *Idh-f* allele in subspp. *alba* and *scotophylla* was expressed only when plants were in active growth.

Viola collina. Only two phenotypes were observed in this species but the geographical pattern was conspicuous. The Lithuanian (population 37) and the Norwegian materials (16 populations) were identical and differed from the German (population 31) by as much as three allelic bands, i.e. in AMP (*b* vs. *be*) and PGM (*ei* vs. *hj*). The *Amp-e* allele was seen also in *V. collina* × *hirta* from Poland (population 69), but this population apparently possessed *Pgm-e* and *Pgm-i*.

Viola hirta. Eleven multilocus phenotypes were found. Variation was detected in AAT (*ae* or *e*), AMP (*cgk*, *cgjk*, or *ck*), IDH (*bch*, *bcfh*, *cdh*, *bchi*, or *bci*), and SKD (*cg*, *g*, or *fg*). No obvious geographical structure was observed, except that allelic diversity was lost towards the north, and that the three Swedish populations (no. 71, 72, 73) did not express *Skd-1* (as seen also in *V. hirta* × *odorata* from population 70). The non-fixed allelic bands *Idh-d*, *Idh-f*, and *Skd-c* were otherwise seen in *V. collina* only, and *Amp-j* only in *V. jaubertiana*.

Viola odorata. Ten multilocus phenotypes were observed. Variation was found in AAT (*be* or *b*), AMP (*fm*, *flm*, *fl*, or *dm*), IDH (*bch* or *bcgh*), PGM (*d* or *c*), and SKD (*e* or *h*). Little geographical structure was found, and the most common phenotype was very widespread and observed in 14 populations, ranging from Tenerife in the south to Norway and Sweden in the north. Interestingly, the Scandinavian

Table 3. All 61 multilocus phenotypes found in the ten *Viola* taxa for 6 enzyme systems. Populations are indicated for each phenotype and asterisks denote populations including more than one multilocus phenotype

Taxon	Population	AAT	AMP	GPI	IDH	PGM	SKD
		a b c d e	a b c d e f g h i j k l m	a b c d e f g h i j	a b c d e f g h i	a b c d e f g h i j k	a b c d e f g h i j
alba ssp.	1 2* 3* 5 6 22	b d	b k	b e	b f h	d h j	d g
alba	3*	b d	b k	b e	b f h	d h j	d i
	2*	b d	b k	b f	b f h	d h j	d g
alba ssp.	1* 5 22	b d	b k	b e	b f h	d h j	d g
scotophylla	1*	b d	b i k	b e	b f h	d h j	d g
alba ssp.	14*	b e	b d k	b f	bc h	d h j	b g
dehnhardtii	7*	b e	b d k	b f	bc h	d j	b d g
	7* 9* 14*	b e	b d k	b f	bc e h	d j	b g
	9*	b e	b d k	b f	bc e	d h j	b g
	7*	b e	b d i k	b f	bc h	d h j	b g
	18	b e	b d i	b f	bc h	d h j	b g
	35	b e	b d i	c f	bc h	d h j	d g
	17	b e	d i	b f	bc h	d h j	b g
	16	b e	d i k	b f	bc e	d h j	b g
	8*	b e	b f i	b f	bc h	d h j	b g
	9*	e	b d k	b f	bc h	d h j	b g
	7*	e	b d k	b f	bc h	d h j	b d g
	7* 8* 15*	e	b d k	b f	bc h	d j	b g
	25*	e	b d k	b f	bc e h	d h j	d g
	25*	e	b d k	b f	bc e	d h j	d g
	12 15*	e	b d i	b f	bc h	d j	b g
	25*	e	b d i	b f	bc e	d h j	d g
	7*	e	b f k	b f	bc h	d h j	b g
	7*	e	b f k	c f	bc h	d h j	d i
ambigua	32	b e	b d g i k	b g i	bc h	a b gh a	f g
collina	37 39 40 42 43 48 49 51 53 55 56 57 58 59 60 63 65a	e	b m	d i	d f h	b e j	c g
	31a	e	b e m	d i	d f h	b h k	c g
hirta	30* a	e	c g k	b j	bc i a	gh	c g
	71 73* a	e	c g k	b j	bc i a	gh	g
	24 a	e	c g k	b j	bc h a	gh	f g

Table 3. (Continued)

Taxon	Population	AAT	AMP	GPI	IDH	PGM	SKD
		a b c d e	a b c d e f g h i j k l m	a b c d e f g h i j	a b c d e f g h i	a b c d e f g h i j k	a b c d e f g h i j
	4* 19 30* 39 40 41 43 57 60* 63 64 65 a	e	c g k	b	j	bc h	a gh	c g
	21 72 a	e	c g k	b	j	bc h	a gh	g
	60* a	e	c g k	b	j	bc f h	a gh	c g
	6 29 44 45 46 66 68 a	e	c g k	b	j	c d h	a gh	c g
	73* a	e	c g k	b	j	bc hi	a gh	g
	30* a	e	c g j k	b	j	bc h	a gh	c g
	4* a	e	c k	b	j	bc h	a gh	c g
	28 a		c g k	b	j	bc i	a gh	f g
jaubertiana	74 a	e	c j	b	i	bc h	d h j	g
odorata	2* 50* b	e	a f l m	b	i	bc gh	d h j	e j
	1 b	e	a f m	b	i	bc gh	c h j	e j
	20* 27 38 47 52 53 54* 55 60* 61 62 63 67 70 75 b	e	a f m	b	i	bc h	d h j	e j
	20* b	e	a f l	b	i	bc gh	d h j	h j
	2* 20* 23 b	e	a f l	b	i	bc gh	d h j	e j
	9 26 50* 54* b	e	a f l	b	i	bc h	d h j	e j
	60* 66 68 b	e	a d m	b	i	bc h	d h j	e j
	15 b		a f l	b	i	bc h	d h j	e j
pyrenaica	10 bc		c m	b	i	a c h	d h j	e
suavis	66 b	e	bc k m	a	hi	bc h	de h j	e g j
	9* b	e	bc k m	b	hi	bc h	b e j k	e g j
	9* 10 11 13* 33 b	e	bc k m	b	hi	bc h	b e h j	e g j
	13* 26 b	e	bc k m	b	hi	bc h	b de h j	e g j
	18 b	e	bc k m	b	hi	bc h	b e j	e g j
	13* b	e	bc k m	a b	hi	a b c h	b e h j	e g j
	13* b	e	bc k m	a b	hi	bc h	b e h j	e g j
	7* b	e	bc k	b	hi	a b c h	b f j k	e g j
	13* b	e	bc k	b	hi	a b c h	b e h j	e g j
	7* b	e	bc k	b	hi	bc h	b e i k	e g j
	8 b	e	bc k	b	hi	bc h	b de h j	e g j
	76 b	e	bc h m	a b	hi	bc h	b de h j	e g j
	36 b		c k m	b	hi	a b c h	b h j	e g j

* variable population

populations had comparatively high levels of variation. The *Amp-l* allele seemed to be more abundant in native populations from southern Europe (no. 2, 9, 15, 20, 23, 26) than elsewhere (no. 50, 54, 63). Noteworthy was also the population from France: Isère (no. 20), with variation in three loci, and three Norwegian populations (no. 60, 66, 68), which expressed the *Amp-d* allele otherwise typical for *V. alba* subsp. *dehnhardtii*.

Viola suavis. This species was enzymatically highly variable but geographic patterns were not seen. Disregarding the dosage differences, which were observed but not interpreted, 13 multilocus phenotypes were found. Variation was found in AAT (*be* or *e*), AMP (*bckm*, *bck*, *bchm*, or *ckm*), GPI (*b*, *ab*, or *a*), IDH (*bch* or *abch*), and PGM (*behi*, *bdehi*, *dehi*, *bei*, *bhi*, *beij*, *bfij*, or *begj*). The Norwegian *V. suavis* population was monomorphic whereas the Mediterranean populations showed high levels of variation. Both *Pgm-e* and *Pgm-j* were shared uniquely with *V. collina*.

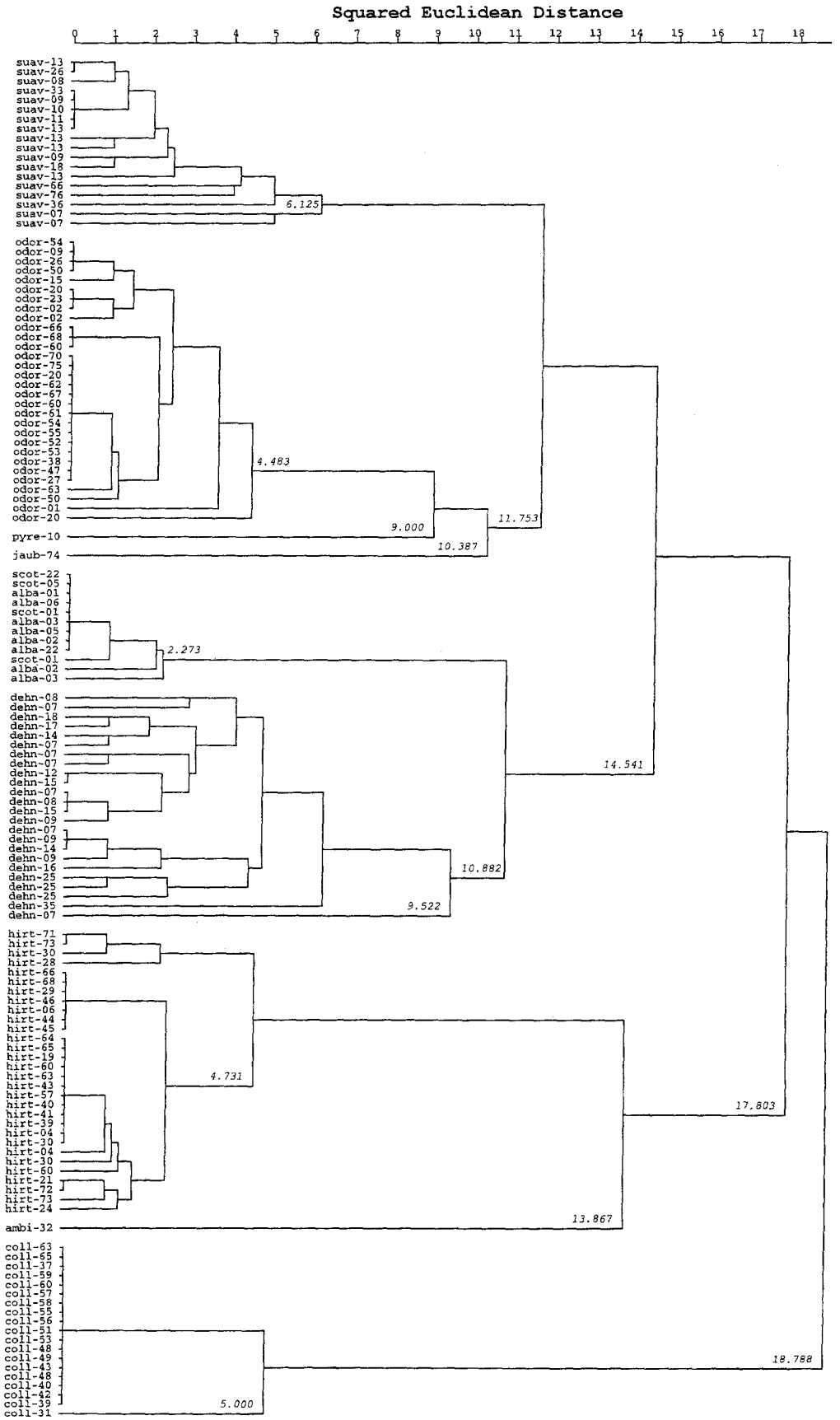
Variation among taxa. Figure 3 presents a UPGMA dendrogram, based on presence-absence of the 58 allozyme markers and the 61 multilocus phenotypes, for each population. The UPGMA did not discriminate the stoloniferous *Flagellatae* series from the estoloniferous *Eflagellatae* series. Four main clusters were recognised. The first comprised *Viola collina* only, the second *V. ambigua* and *V. hirta*, the third *V. alba* and its subspecies, and the fourth comprised *V. jaubertiana*, *V. odorata*, *V. pyrenaica*, and *V. suavis*. Except within *V. alba*, clusters were remarkably distinct and in agreement with current taxon circumscriptions. *V. alba* subspp. *alba* and *scotophylla* formed one mixed cluster, showing that these taxa cannot be identified on the basis of allozymes, whereas subsp. *dehnhardtii* was distinct and variable.

Only minor changes in tree topology, notably regarding the placement of *Viola jaubertiana*, occur when other clustering methods or distance measures were used, or when the two 2n = 40 taxa, *V. ambigua* and *V. suavis*, were removed from the analyses.

The PCO plot (Fig. 4) (3 first axes) was based on the 58 enzyme markers and the collective phenotypes (all allelic bands of a single taxon pooled in a single phenotype) for all ten *Viola* taxa. PCO axes 1, 2, and 3 extracted 23.5%, 20.6%, and 14.7% of the variance, respectively. Taxa were well spread but only poor separation was obtained for *V. alba* subspp. *alba* and *scotophylla*. Axis 1 separated *V. alba* from the other taxa, axis 2 separated *V. ambigua*, *V. collina*, and *V. hirta*, and axis 3 separated *V. collina*. Noteworthy was the position of *V. suavis* (2n = 40) close to *V. jaubertiana*, *V. odorata*, and *V. pyrenaica*; and, also, *V. ambigua* (2n = 40) in the proximity of *V. hirta*. The main UPGMA clusters were recognised and, like the UPGMA, the PCO failed to separate series *Flagellatae* (gray pinheads) and *Eflagellatae* (white pinheads). However, the flagellate taxa tended to cluster in the centre of the plot and the eflagellate to take more peripheral positions.

Table 4 shows the percentage of bands shared by each of the 2n = 20 taxa and the two 2n = 40 taxa, *Viola ambigua* and *V. suavis*. *V. jaubertiana* (62%), *V. hirta* (60%), and *V. alba* subsp. *scotophylla* (60%), shared the highest proportions of allelic bands with *V. ambigua*; *V. collina* (41%) and *V. odorata* (42%) the lowest. It should be pointed out that *V. ambigua* shared three unique alleles with *V. hirta* (*Amp-g*, *Pgm-a*, and *Skd-f*), one with *V. alba* subsp. *dehnhardtii* (*Amp-d*), and none with the other taxa. *V. pyrenaica* (92%), *V. jaubertiana* (85%), and *V. odorata* (68%) shared the highest proportion of bands with *V. suavis*; *V. hirta* the lowest (50%). However, *V. suavis* shared two unique bands with *V. collina* (*Pgm-e* and *Pgm-k*), one with *V. pyrenaica* (*Idh-a*), and one with *V. odorata* (*Skd-j*).

The stolon character, commonly used in the subdivision of the subsection, discriminated well among the species (Fig. 5). Not surprisingly, long internodes and stolons were found in the *Flagellatae* species only, i.e. *V. alba*, *V. jaubertiana*, *V. odorata*, and *V. suavis*. On the other hand, the internodes in the *Eflagel-*


Squared Euclidean Distance
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
suav-13
suav-26
suav-08
suav-33
suav-09
suav-10
suav-11
suav-13
suav-13
suav-13
suav-09
suav-18
suav-13
suav-66
suav-76
suav-36
suav-07
suav-07
6.125
odor-54
odor-09
odor-26
odor-50
odor-15
odor-20
odor-23
odor-02
odor-02
odor-66
odor-68
odor-60
odor-70
odor-75
odor-20
odor-62
odor-67
odor-60
odor-61
odor-54
odor-55
odor-52
odor-53
odor-38
odor-47
odor-27
odor-63
odor-50
odor-01
odor-20
4.483
9.000
11.753
pyre-10
jaub-74
10.387
scot-22
scot-05
alba-01
alba-06
scot-01
alba-03
alba-05
alba-02
alba-22
scot-01
alba-02
alba-03
2.273
dehn-08
dehn-07
dehn-18
dehn-17
dehn-14
dehn-07
dehn-07
dehn-07
dehn-12
dehn-15
dehn-07
dehn-08
dehn-15
dehn-09
dehn-07
dehn-09
dehn-14
dehn-09
dehn-16
dehn-25
dehn-25
dehn-25
dehn-35
dehn-07
14.541
10.882
9.522
hirt-71
hirt-73
hirt-30
hirt-28
hirt-66
hirt-68
hirt-29
hirt-46
hirt-06
hirt-44
hirt-45
hirt-64
hirt-65
hirt-19
hirt-60
hirt-63
hirt-43
hirt-57
hirt-40
hirt-41
hirt-39
hirt-04
hirt-30
hirt-04
hirt-30
hirt-60
hirt-21
hirt-72
hirt-73
hirt-24
4.731
17.803
13.867
ambi-32
coll-63
coll-65
coll-37
coll-59
coll-60
coll-57
coll-58
coll-55
coll-56
coll-51
coll-53
coll-48
coll-49
coll-43
coll-48
coll-40
coll-42
coll-39
coll-31
18.788
5.000

latae species were either entirely absent (*V. ambigua*, *V. hirta*, and *V. pyrenaica*) or reduced (*V. collina*). Hybrids involving stoloniferous taxa were usually stoloniferous as well (see below).

Characterisation of the putative hybrids. Compared to the putative F_1 hybrids, identified by full band additivity with the parental species, later-generation hybrids showed a marked reduction in heterozygosity per generation (Table 5), on average 0.51.

Stolons were either dominantly or codominantly inherited in the hybrids (Fig. 5). Only *Viola collina* × *hirta* was estoloniferous. *V. alba* × *hirta* and *V. alba* × *odorata* were similar to *V. alba* in this character, whereas

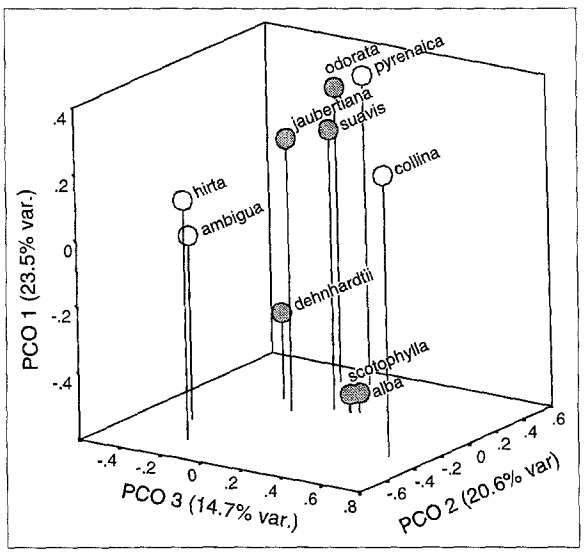


Fig. 4. PCO plot (3 first axes) based on presence/absence of 58 enzyme markers and collective phenotypes (pooling all allelic bands in a single taxon) for the ten *Viola* taxa. The series *Eflagellatae* (gray pinheads) and *Flagellatae* (white pinheads) are indicated. PCO axes 1, 2, and 3 extracted 23.5%, 20.6%, and 14.7% of the variance, respectively

Fig. 3. UPGMA dendrogram based on presence-absence of 58 allozyme markers and multilocus phenotypes for each population of *Viola* (Tables 2 and 3). Squared Euclidean Distance was applied. Taxon and population number are shown

Table 4. Percentage of allelic bands shared between each of the 2n = 20 taxa and the two 2n = 40 taxa, *Viola ambigua* and *V. suavis*

2n = 20 taxa	Percentage of allelic bands shared with the 2n = 40 taxa	
	V. ambigua	*V. suavis*
V. alba ssp. *alba*	53	67
V. alba ssp. *dehnhardtii*	57	57
V. alba ssp. *scotophylla*	60*	67
V. collina	41	65
V. hirta	60*	50
V. jaubertiana	62*	85*
V. odorata	42	68
V. pyrenaica	46	92*

* Highest percentage of band sharing

V. collina × *odorata* was similar to *V. odorata*. *V. collina* × *hirta* and *V. hirta* × *odorata* were intermediate between the parental species. Segregation of morphological characters, including the stolon character, was the rule in F_2 and F_3 hybrids. Most later-generation hybrids were vigourous, whereas a few were weak and rapidly died.

F_1 hybrids were always distinctly less fertile than the parental species. Numerous aborted capsules or capsules with 1–3(4) seeds were produced, versus (10)15–30(40) seeds per capsule in the parental species. Both average fertility and fertility variance increased in F_2 and F_3 hybrids, and the F_n and F_{n+1} hybrids usually had fertilities approaching the parental species (Fig. 6). However, some vigourous F_2 individuals failed to produce capsules, although cultivated for more than a year.

Viola alba × *hirta*. The putative F_1 hybrids displayed heterosis and approached *V. alba* in stolon development. The F_1 hybrids were moderately fertile (mode 1.4 seed per capsule), whereas the F_2 hybrids displayed considerably higher fertilities than any other F_2 hybrid combinations (1.5–9.5 seeds per capsule; Fig. 6).

Viola alba × *odorata*. The putative F_1 hybrids were generally similar to *V. alba* but more vigourous, producing numerous long

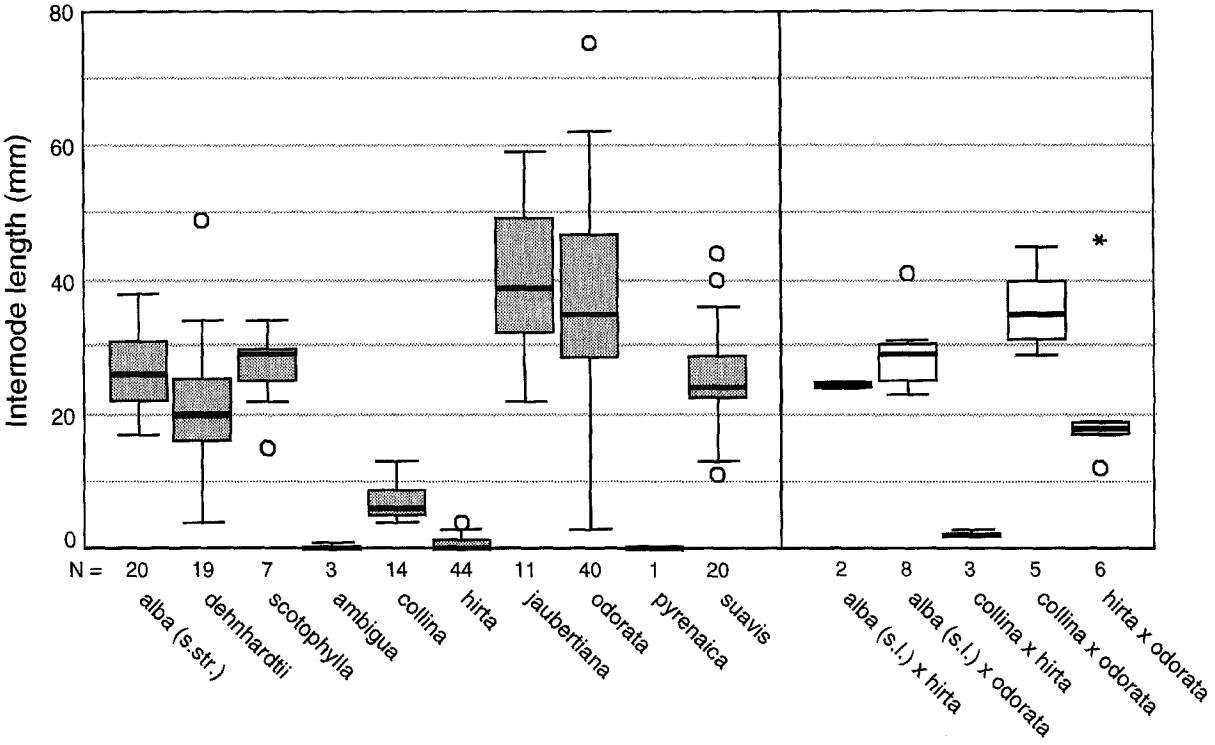


Fig. 5. Boxplot of maximal internode length on lateral shoots (stolons) of *Viola alba* subsp. *alba*, subsp. *dehnhardtii*, subsp. *scotophylla*, *V. ambigua*, *V. collina*, *V. hirta*, *V. jaubertiana*, *V. odorata*, *V. pyrenaica*, *V. suavis* (gray boxes), and five F_1 hybrid combinations (white boxes). Outliers (open circles), far outliers (asterisks), and number of individuals (N) are indicated for each taxon

stolons. Also the F_n hybrids were most similar to *V. alba* but possessed some aberrant features pointing in the direction of *V. odorata* (e.g. in stipule characters). The F_1 hybrids had the lowest average fertility among the different hybrid combinations (mode 1.0 seeds per capsule), and usually failed to set seeds. However, an outlier with 2.2 seeds per capsule is noteworthy (population 15). The F_n and F_{n+1} hybrids all had fertilities similar to the parental species (9.0–26.5 seeds per capsule; Fig. 6).

Viola collina × *hirta*. The putative F_1 hybrids were roughly intermediate between the parental species, and little heterosis was expressed in the cultivated material. Whereas the F_2 and F_3 hybrids showed some segregation of morphological characters, the F_n and F_{n+1} hybrids (population 69) were difficult to discern from the variable *V. hirta*. However, increased vegetative pigmentation, stipule fimbrication, and internode length disclosed the presence of *collina* genes in these hybrid derivativs. The F_1 hybrids were moderately fertile (mode 1.4 seeds per capsule), and the F_2 hybrids showed modest increases in fertility (1.2–3.3 seeds per capsule). A further increased fertility was found in the F_3 hybrids, of which one individual had attained normal fertility (3.6–15.8 seeds per capsule). While F_n hybrids had normal fertility, the F_{n+1} hybrids surprisingly showed significant decreases in fertility, most likely a consequence of cultivation conditions (Fig. 6).

Viola collina × *odorata*. The putative F_1 hybrids were stoloniferous and approached *V. odorata* in general morphology. Heterosis was expressed in several characters. The F_1 hybrids were moderately fertile (mode 1.3 seeds per capsule) but differences in fertility among populations were observed (cf. the upper outlier with 1.6 seeds per capsule from

Table 5. Multilocus phenotypes found among *Viola* hybrids for 16 putative loci. All hybrids showing full additivity with the parental species are interpreted as F_1 hybrids. Hybrid combination, generation, population, and individual are specified for each multilocus phenotype

Taxon	Generation, population, individual	AAT					AMP											GPI									IDH								PGM									SKD								
		a	b	c	d	e	a	b	c	d	e	f	g	h	i	j	k	l	m	a	b	c	d	e	f	g	h	i	j	a	b	c	d	e	f	g	h	i	a	b	c	d	e	f	g	h	i	j	k	a	b	c	d	e	f	g	h	i	j
alba × *hirta*	F_1 3-1	a	b		d	e		b	c				g					k			b		e					j		b	c			f			h	i	a		d			g	h		j			c	d			g				
	F_2 3-1-1	a	b		d	e		b	c				g					k			b		e							b	c			f			h	i			d			g	h		j				d			g				
	F_2 3-1-2		b			e		b	c				g					k			b		e							b	c			f			h	i	a		d			g	h					c	d			g				
alba × *odorata*	F_1 2-1		b		d	e	a	b				f						k	l		b				f			i		b	c				f	g	h				d				h		j				d	e		g		j
dehnhardtii × *odorata*	F_1 34-1		b			e		b		d		f						k	l		b				f			i		b	c						h				d				h		j		b			e		g		j
	F_1 15-1		b			e	a	b		d		f						k	l		b				f			i		b	c						h				d				h		j		b			e		g		j
	F_1 15-2		b			e	a	b		d		f						k	l		b				f			i		b	c		e				h				d				h		j		b			e		g		j
	F_1 15-3		b			e	a	b		d		f			i			l			b				f			i		b	c						h				d				h		j		b			e		g		j
	F_n 15-4		b			e		b		d					i										f					b	c		e				h				d				h		j		b							j
	F_n 15-5		b			e		b				f						k			b				f					b	c						h				d				h		j					e		g		j
collina × *hirta*	F_1 (all)	a				e		b	c				g					k	m		b		d					i	j	b	c	d		f			h		a	b			e		g	h		j			c				g				
	F_2 60-1-1	a				e		b	c				g					k	m		b		d					i	j	b	c	d		f			h		a				e		g	h		j			c				g				
	F_2 60-1-2	a				e		b	c									k	m				d					i	j		c	d		f			h		a	b			e		g	h		j			c				g				
	F_3 60-1-2-1	a				e		b										k					d					i			c	d		f			h			b			e		g			j			c				g				
	F_3 60-1-2-2	a				e		b	c									k	m				d					i	j			d		f			h			b			e		g			j			c				g				
	F_n 69-1	a				e		b	c		e							k	m		b		d						j	b	c			f			h		a						g			j			c				g				
	F_n 69-2	a				e		b	c		e							k	m		b		d					i	j	b	c			f			h			b						g			j			c				g				
	F_n 69-3	a				e		b			e							k	m		b							i		b	c			f			h			b						g	h		j			c				g				
	F_{n+1} 69-1-1	a				e		b			e							k			b								j	b	c			f			h		a						g			j			c				g				
	F_{n+1} 69-1-2	a				e		b	c		e							k	m		b		d						j	b	c			f			h		a						g			j			c				g				
	F_{n+1} 69-1-3	a				e		b	c		e							k			b		d						j	b	c			f			h		a						g			j			c				g				
	F_{n+1} 69-1-4	a				e		b			e							k			b								j	b	c			f			h		a						g			j			c				g				
	F_{n+1} 69-1-5	a				e		b	c										m			b		d						j	b				f			h		a						g			j			c				g				
	F_{n+1} 69-1-6	a				e		b	c		e							k	m		b		d						j	b	c			f			h		a						g			j			c				g				
	F_{n+1} 69-1-7	a				e		b	c		e								m		b								j	b	c			f			h		a									j			c				g				
	F_{n+1} 69-3-1	a				e		b			e							k			b							i		b	c			f			h			b						g	h		j			c				g				
	F_{n+1} 69-3-2	a				e		b			e							k	m		b							i		b	c			f			h			b						g	h		j			c				g				
	F_{n+1} 69-3-3	a				e		b			e							k			b							i		b	c						h			b						g	h		j			c				g				
collina × *odorata*	F_1 (63)	a	b			e	a	b				f							l	m	b		d					i		b	c	d		f			h			b		d	e			h		j			c		e		g			j
	F_1 (53 55 60)	a	b			e	a	b				f								m	b		d					i		b	c	d		f			h			b		d	e			h		j			c		e		g			j
	F_2 60-2-1		b			e		b				f								m	b							i		b	c	d		f			h			b		d	e			h		j					e		g			j
	F_2 60-2-2	a	b			e	a	b				f								m	b		d					i				d		f			h					d	e					j			c		e		g			j

Table 5. (Continued)

Taxon	Generation, population, individual	AAT				AMP										GPI										IDH										PGM										SKD									
		a	b	c	d	e	a	b	c	d	e	f	g	h	i	j	k	l	m	a	b	c	d	e	f	g	h	i	j	a	b	c	d	e	f	g	h	i	j	k	a	b	c	d	e	f	g	h	i	j
hirta × *odorata*	F_1 20*	a	b			e	a		c			f	g				k	l		b									i	j	b	c					h	i		a		d			g	h	j		c		e		g		j
	F_1 20*	a	b			e	a		c			f	g				k	l		b									i	j	b	c					h			a		d			g	h	j				e			h	j
	F_1 23-1	a	b			e	a		c			f	g				k	l		b									i	j	b	c					g	h		a		d			g	h	j				e		g		j
	F_1 60-3 (63)	a	b			e	a		c			f	g				k	l		b									i	j	b	c					h			a		d			g	h	j				c	e	g		j
	F_1 60-4 (63 70)	a	b			e	a		c			f	g				k	m		b									i	j	b	c					h			a		d			g	h	j				c	e	g		j
	F_1 68-1	a	b			e	a			d		f	g				k	m		b									i	j	b	c	d				h			a		d			g	h	j				c	e	g		j
	F_2 23-1-1		b			e	a		c				g				l			b									i		b	c					g	h							h	j					e	g		j
	F_2 23-1-2		b			e	a		c				g				k	l		b									i	j	b	c					h			a		d			g	h	j				e			j
	F_2 60-4-1		b			e	a		c				g					m		b									i	j	b	c					h			a		d			h	j				c	e	g		j
	F_2 60-4-2	a	b			e			c				g				k			b									i	j	b	c					h			a		d			g	h					c		g		
	F_2 60-4-3	a	b			e	a						g					m		b									i		b	c					h			a		d			h	j					c				j
	F_2 68-1-1	a	b			e	a			d		f	g				k			b									i	j	b	c					h			a					h	j					c	e			j
	F_2 68-1-2		b			e	a					f	g				k	m		b									i			c	d				h			a		d			h	j					c	e	g		j

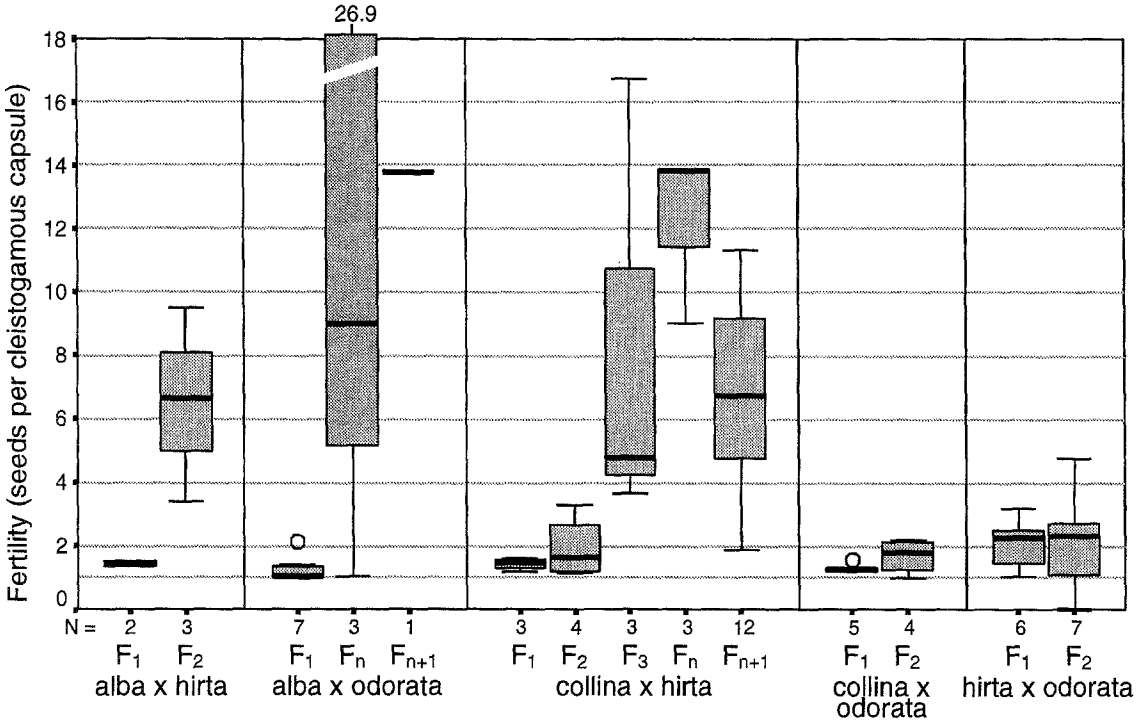


Fig. 6. Boxplots of hybrid fertility, measured as seeds per capsule for different generations of five *Viola* hybrids and based on an average 21.3 ± 14.8 capsules per individual. The late-generation hybrids (F_n and F_{n+1}) of *V. alba* × *odorata* and *V. collina* × *hirta* have fertilities similar to their parental species

volved in the origin of section *Viola* have belonged to different evolutionary lineages. The entire section *Viola* thus seems to be paleotetraploid and of alloploid origin (cf. Marcussen and Nordal 1998, Nordal and Jonsell 1998). However, evolutionary events subsequent to the polyploid origin, e.g. mutations and gene silencing, may also have contributed to the current genomic distance. In any case, the fact that only two alleles were shared by all taxa, indicates an old ancestry of subsection *Viola* (cf. Crawford 1989). In the two octoploids (2n = 40), *V. ambigua* and *V. suavis*, a large proportion of fixed heterozygosity indicates alloploid origins, presumably from more recent hybridisation between tetraploid taxa within subsection *Viola*.

Variation patterns. So far, most studies on temperate, cleistogamous violets have revealed low levels of enzyme variation, i.e. within the *V. albida* complex, section *Adnatae*, in Korea (Kim et al. 1991), in European *V. rupestris* (Nordal and Jonsell 1998) and *V. elatior* (A. Gyrax unpublished), both in section *Viola*, and in North American *V. canadensis*, section *Canadenses* (T. Culley submitted). However, one study on diploid *V. pubescens*, section *Chamaemelanium*, in North America shows high levels of variation and heterozygosity, which suggests a more prevalent role of chasmogamous reproduction (Culley and Wolfe 2000).

Our data show that most populations of the taxa within subsection *Viola* in northern Europe are depleted of variation but that much more variation is occasionally present in Mediterranean taxa, notably *V. alba* subsp. *dehnhardtii* and *V. suavis*. There are some obvious reasons for this pattern.

First, genetic variation is expected to decrease with increasing distance from glacial refugia (Barrett and Kohn 1991, Taberlet 1998). Notably, important glacial refugia for the Eurasian nemoral flora, violets included, are postulated in a narrow belt from the Mediterranean area eastwards to the Caucasus

and North Iran (Polunin and Walters 1985, Fukarek 1995, Taberlet 1998, Taberlet et al. 1998). The investigated Mediterranean populations grow fairly close to these postulated refugia, and high levels of variation in Mediterranean taxa are thus in accordance with the predictions. Furthermore, consecutive founder events along the expanding migration front will result in only a small subset of the original variation being retained in peripheral populations like those in North Europe. Current levels of intraspecific variation can, however, have other historical reasons, such as genetic bottlenecks or past introgressive hybridisation.

Second, resource allocation to chasmogamy versus cleistogamy among species of subsection *Viola* seems to differ in northern and southern Europe. Peak chasmogamous flowering takes place in early spring, and low pollinator activity and availability may be a serious constraint to chasmogamous seed set in the north (unpublished data). Since low chasmogamous seed output is buffered by increased cleistogamous seed output (Redbo-Torstensson and Berg 1995), increased inbreeding may affect the levels of genetic variation, particularly within populations of the earliest-flowering species. Indeed, segregating heterozygotes appear to be considerably rarer in the otherwise variable *V. alba* subsp. *dehnhardtii* and *V. suavis* than in a largely outcrossing species such as *V. pubescens* (Culley and Wolfe 2000), indicating a higher allocation to cleistogamy and inbreeding in subsection *Viola*, resulting in levels of heterozygosity comparable to most other violets studied (Kim et al. 1991, Nordal and Jonsell 1998, Culley submitted).

Some of the observed variation is due to lack of bands, which can result from co-migration with other gene products or temporal or permanent inactivation of the gene in question or its gene product. In polyploids, non-functional alleles may be less disadvantageous than in diploids because other functional alleles are present. Null alleles can therefore be maintained as polymorphisms or, eventually, be fixed and contribute to the diploidisation of the genome (Soltis and Soltis 1989, 1999; Werth 1989; Wendel 1999). However, several studies have shown that isozyme expression in plants is also affected by developmental stage and ecological conditions (Mowrey and Werner 1990, Asins et al. 1993, Asins et al. 1995). In this study, *Idh-3* in *V. alba* subspp. *alba* and *scotophylla* was expressed only when plants were in active growth, and the same could apply to apparently silenced loci in the other taxa as well.

Viola alba. The two sympatric subspecies, *alba* and *scotophylla*, cannot be distinguished by means of allozymes. The morphological distinction between *alba* and *scotophylla* is in anthocyan pigmentation only (Becker 1910): Subsp. *scotophylla* is usually strongly pigmented in all vegetative parts (*scotophylla* means 'dark-leaved'), while *alba* is not. Floral albinism is prevalent in both *alba* and *scotophylla*; only one population of *scotophylla* (no. 1) had pigmented corollas. Pigmentation is often controlled by few genes, and in our opinion, the *alba* morph is probably derived from a pigmented, *scotophylla*-like, ancestor by loss of pigment expression. We therefore suggest that *scotophylla* is treated merely as a form of *V. alba* subsp. *alba*. Considering reconstructed phylogeographies for various European plant and animal species (e.g. Demesure et al. 1996, Taberlet et al. 1998) and the distribution of the *V. alba* complex, a main glacial refugium for both *alba* and *scotophylla* seems to have been in the Caucasus. Hence, the gradual increase in the frequency of the *alba* morph north and west of the Alps could result from random events during post-glacial migration.

The parapatric subsp. *dehnhardtii*, on the other hand, is distinct, both with respect to allozymes and morphology. In *dehnhardtii*, floral albinism is infrequent but vegetative parts are usually strongly pigmented. A main reason for the high level of genetic diversity within *dehnhardtii* is consistent with a possible survival in refugia in the Mediterranean region throughout the Quarternary.

A transition zone between *alba*/*scotophylla* and *dehnhardtii*, presumably a persistent hy-

brid zone (cf. Arnold 1997, Arnold et al. 1999), stretches across southern Europe (Becker 1910, Strid 1986, Muñoz Garmendia et al. 1993). Some materials of *dehnhardtii* from France (e.g. population 7) possessed a few *alba* alleles and may represent the southern boundary of this transition zone.

Viola collina. Quite unexpectedly, all materials from Norway and Lithuania lacked enzyme variation. Southeast Norway is the only region in northern Europe where the widespread *V. collina* has a continuous distribution and is relatively frequent. Moreover, phytogeography (Nordal 1996) indicates that the Norwegian populations represent the westernmost remnants of a previously much wider, post-glacial distribution in Fennoscandia, and, most likely, *V. collina* was an early post-glacial immigrant during the warm, dry, continental climatic periods of the Hypsithermal (7,500–2,500 Y. B. P.) (cf. Fægri 1996). Allozymes suggest that *V. collina* colonised the Nordic countries from the east, and that it was recruited from other glacial refugia than the Central European populations, here represented by the deviating population from Germany: Harz (no. 31), and the hybrid population from Poland: Poznan (no. 69). *V. collina* is shown to reproduce chiefly by cleistogamy in Scandinavia (unpublished data), which may contribute to within-population depletion of genetic variation. However, the total lack of variation among populations in Norway suggests a single introduction, combined with severe genetic bottlenecks.

Viola hirta. Our data show a cline of genetic depletion northwards, and the northwestern populations, i.e. those in Norway, show no variation at all. Norwegian populations are isolated and located in the outskirts of the main distribution area, quite distant from putative glacial refugia and from other populations in northern Europe, i.e. in southern Sweden. Both circumstances may have a strong effect on genetic variation (cf. Barrett and Kohn 1991, Marcussen et al. submitted).

Based on stipule characters, Becker (1910, 1918) split *V. hirta* in two subspecies: the Central and North European subsp. *hirta* (i.e. subsp. *brevifimbriata* W. Becker) and the Southeast European and Caucasian subsp. *longifimbriata* W. Becker. Becker (1918) noted that the latter approached *V. collina* in morphology, and Gerstlauer (1943) indicated that the long-fimbriate morph could indeed have originated by introgression from *V. collina.* Our material from Poland: Poznan (population 69) apparently represents late-generation hybrids between *V. collina* and *V. hirta* and comes close to Becker's subsp. *longifimbriata* in having pronounced pubescence and long-fimbriate stipules. We thus consider subsp. *longifimbriata* as a hybrid product between *V. collina* and *V. hirta*, not a geographical race as proposed by Becker (1918). Most likely, this morph can therefore occur not only in southern Europe (cf. Becker 1910) but also elsewhere, if the two parental species meet.

Viola jaubertiana. This species is enzymatic as well as morphologically quite distinct and should not be included in *V. alba* as suggested by Becker (1910). Its glabrousness and strongly increased epidermal cell size (Tchourina 1909), endemic distribution in Majorca (Chodat 1924), and its highly sterile hybrid with *V. alba* gave Schmidt (1961) reasons to believe that *V. jaubertiana* represents a relictual and isolated lineage among European members of subsection *Viola.* This hypothesis is supported also by allozymes, as most of the allelic bands observed in *V. jaubertiana* are widespread in the subsection as well as in the entire section (unpublished data), and the species shows no strong affinity to other taxa. This implies that these alleles may be primitive and, hence, that any enzymatic similarities with other taxa are plesiomorphic.

Viola odorata. This species does not occur natively in the northern parts of Europe. Nevertheless, it is now widespread due to escape from cultivation, both as an ornamental and as a source of essential oils for the perfume industry. Our data strongly support multiple introductions to Norway, as indicated by a far higher amount of enzymatic diversity than in the native species. Variable fertility in hybrid

combinations, particularly with *V. hirta*, further suggests genetic differentiation in *V. odorata* in northern Europe and, thus, recruitment from different source populations.

The population from France: Isère (no. 21) deviates morphologically (more hairy, paler flowers), ecologically (occurring in steep, grassy slopes at the edges of boreal forests), and enzymatic (one unique allele, *Skd-h*). These facts all point to the population being a possible remnant of native *V. odorata*, as yet little affected by introgression from the far more common *V. odorata* cultivars, which otherwise seem to dominate throughout much of Europe.

Viola suavis. Historically, this enzymatically variable species has been cultivated to a lesser extent than *V. odorata* but its use as an ornamental has probably expanded its distribution area, especially in Central Europe and in parts of the Iberian Peninsula (Yuzepchuk et al. 1949, Muñoz Garmendia et al. 1993, Marcussen and Nordal 1998). Consequently, only the eight French populations (no. 7, 8, 9, 10, 11, 13, 18, 26) are probably truly native and apparently include numerous morphs or ecotypes, both vigourous anthropochorous types and more specialised, native types. Neither of these were, however, enzymatically recognisable. A fuller account of the variation in *V. suavis* is presented in Marcussen and Nordal (1998).

Reticulate evolution. The ability of frequent hybridisation is characteristic for most groups of violets (cf. Fig. 1), and it seems likely that this trait has had major impact on evolutionary pathways and current relationships in the genus (cf. Rieseberg and Ellstrand 1993, Arnold et al. 1999). Hybridisation and introgression are facilitated by the lack of internal crossing barriers and probably accelerated by the breakdown of ecological and geographical barriers caused by past and present human activity (Schöfer 1954, Moore 1959, Schmidt 1961, Gil-ad 1997, Neuffer et al. 1999). Furthermore, most species within subsection *Viola* share pollinators and have largely overlapping chasmogamous flowering periods and habitat requirements (Beattie 1969a, b, 1971, 1972, 1974; Hiemeyer 1992; Nordal 1996).

The potential for alloploid speciation in *Viola* is illustrated by the experiments of Dodd and Gershoy (1934), who successfully produced a fully fertile and vigourous dodecaploid (2n = 60) from the sterile, intersubsectional hybrid *V. odorata* × *riviniana* (2n = 30). The present study further suggests that one or more polyploid events took place early in the evolutionary history of section *Viola*, and that this section originated through fusion of different diploid lineages by alloploidy. Apparently, also recombinant homoploid speciation has contributed to the stabilisation of hybrids in *Viola* (Moore 1959, Valentine 1962, Gil-ad 1997), and it seems likely that cleistogamous inbreeding has played an important role in the stabilisation process. However, hybridisation and introgression in subsection *Viola* are retarded by low hybrid fertility, particularly in northern Europe, where chasmogamous seed set is low and the amount of hybrid seeds negligible compared to the vast amount of cleistogamous seeds produced by the parental taxa (Beattie 1969b, 1972; Redbo-Torstensson and Berg 1995; Banasinska and Kuta 1996). Also, hybrid vigour and fertility in the next generations are crucial, but unpredictable. For instance, the F_2 hybrids of *V. alba* × *hirta* showed pronounced increase in fertility while apparent hybrid breakdown was found among the F_2's of *V. hirta* × *odorata*, which failed to produce flowers in cultivation.

Nevertheless, this study shows that several hybrid generations can be raised from seeds, and spontaneously occurring hybrid derivatives are discovered for *V. alba* sp. *dehnhardtii* × *odorata* (population 15) and *V. collina* × *hirta* (population 69). Furthermore, putative ancient allele transfers, maintained as polymorphisms, are traced from *V. alba* subsp. *dehnhardtii* to *V. odorata* (*Amp-d*) and vice-versa (*Amp-f*), and from *V. collina* to *V. hirta* (*Idh-d*, *Idh-f*, *Skd-c*). Notably, gene exchange has taken place between *V. alba* subsp. *dehnhardtii* and *V. odorata* in spite of the high infertility of their

primary hybrid (cf. Schmidt 1961). These findings imply that overlap in geographic distributions and ecological demands in the parental species, along with vigour and vegetative spread in their hybrids, may overcome constraints imposed by e.g. low hybrid fertility and hybrid breakdown (cf. Rieseberg and Ellstrand 1993, Arnold et al. 1999).

Thus, given the time and the opportunity, potentials for introgression and hybrid speciation are present in subsection *Viola*. In the history of the subsection, hybrids have apparently been stabilised mainly at the homoploidal level (2n = 20) but occasional, additional polyploidisation has taken place.

The distribution of allelic bands in subsection *Viola* is in many cases incompatible with the overall structure, and several taxa share rare bands with other taxa than those they cluster with in the UPGMA or the PCO. Although shared allozyme bands usually reflect relationships among taxa, patterns may become considerably obscured by vertical or horizontal transfer of alleles in the history of a group. Alleles that are passed on vertically to derived taxa may result in independent fixation of identical alleles in remotely related taxa (plesiomorphic alleles). Some of the contradictory allele distributions are probably also due to recurrent introgressive hybridisation and horizontal allele transfer among taxa. Introgressed alleles may subsequently get fixed in populations or even in entire taxa or lineages.

Successive events of isolation, speciation, migration, hybridisation, and stabilisation of hybrid products enforced by Pleistocene climatic fluctuations have probably been important in the evolution within the subsection (Valentine 1962, Gil-ad 1997). Recurrent isolation and secondary contact among species may have allowed taxa, modified by hybridisation, to spread and occupy new habitats. Both repeated migrations and, more recently, the modifying action of man on habitats have probably facilitated hybridisation (cf. Valentine 1962, Neuffer et al. 1999). The notoriously weak internal isolation barriers have certainly been important in the initial establishment of hybrid swarms, and the ability to exchange genes may have provided taxa with possibilities to respond quickly to environmental changes. Extant taxa may thus be viewed as temporarily stabilised genotypes of hybrid origin, with a potential for future gene flow across species boundaries. In this perspective, taxonomic entities may be relatively short-lived in subsection *Viola*.

A reticulate history of evolution may partly explain the difficulties in tracing the origins of the octoploids, *V. ambigua* and *V. suavis*, among extant tetraploid taxa. However, the two octoploids do have stronger affinities to some of the tetraploids than to others.

Viola ambigua seems most closely related to *V. hirta*. Whereas the bands shared with *V. odorata* and *V. alba* subsp. *dehnhardtii* are common within the subsection, as many as three unique bands are shared by *V. ambigua* and *V. hirta*. The morphological delimitation of *V. ambigua* from *V. hirta* has, indeed, been confusing, as the two species also have overlapping distributions and habitat requirements (Gerstlauer 1943, Yuzepchuk 1949, Schmidt 1961). Moreover, cytological investigations of their hybrid indicate that they have one genome in common, and the hybrid is moderately fertile (Schmidt 1961). Nevertheless, present allozyme patterns do not, by any means, suggest *V. hirta* as a direct or recent parent of *V. ambigua*.

Viola suavis seems particularly closely related to *V. pyrenaica*. As much as 92% of the *pyrenaica* markers are shared with *V. suavis* and, combined with similarities in morphology, *V. pyrenaica* is suggested as one parent for *V. suavis*. Its second parent, however, is far less evident, and close relationships with both *V. jaubertiana*, *V. odorata*, and *V. collina* are indicated. Whereas bands shared with the two first species mainly are common ones in the subsection, two unique bands are shared with *V. collina*. On the other hand, a close relationship with *V. odorata* is supported by slight hybrid fertility (Schnarf 1922) whereas some morphological traits point towards *V. jaubertiana* (cf. Marcussen and Nordal 1998).

The failure to fully trace the origin of *V. suavis*, combined with its large enzymatic and morphological variability and wide ecological amplitude, may suggest an old age of this species. Also, as multiple origins are the rule in polyploids (Soltis and Soltis 1993, 1999), it seems likely that *V. suavis* has originated recurrently from different parental populations (polytopy) or even from different parental taxa (polyphyly). Subsequent gene flow among these nascent octoploids is also likely to have happened, along with gene flow among octoploids and related tetraploids (Petit et al. 1999, Soltis and Soltis 1999, Wendel 1999).

The bibracteolate stolons of subsection *Viola* are, most likely, homologous with the unibracteolate aerial, floriferous stems in members of more primitive groups, including subsection *Rostratae* (Clausen 1929, 1962; Ballard et al. 1999). Stolons may therefore be a primitive character in subsection *Viola*, while reduction or absence is derived. Allozyme data partly support this hypothesis. The PCO plot places the stoloniferous taxa in the centre, indicating relatively undifferentiated allozyme phenotypes. *V. jaubertiana* in particular, with its peculiar morphology and relictual distribution, may come close to the root of the subsection.

Interspecific hybridisation occurs between as well as within the two series (Fig. 1), and considerable inter-serial gene flow may have taken place in the evolutionary history of the subsection. Allozyme data (Figs. 3, 4) further obscure series delimitation and suggest that the reduction of stolons has occurred more than once. Different inheritance of the stolon character among the various hybrids (Fig. 5) indicates different genetic control in the parental taxa. Moreover, stolons are occasionally rudimental or not present at all in the usually stoloniferous *V. alba* subsp. *dehnhardtii* (Becker 1910, Valentine et al. 1968) and *V. suavis* (pers. obs.), i.e., suppression of stolon development may have originated independently even within extant taxa. Hence, the subdivision into series *Flagellatae* and *Eflagellatae* probably does not reflect true phylogenetic relationships but merely an evolutionary trend that causes variation in a single macromorphological character well suited to differentiate among taxa.

Conclusions

Our isozyme studies indicate that *Viola* subsection *Viola* represents a polyploid complex of ancient hybrid origin, where extant taxa are either tetraploids or octoploids. A general lack of internal crossing barriers has facilitated hybridisation. In Europe, instability caused by Quartenary glaciations and, later, human activity have resulted in extensive contact between lineages. Occasional introgression may have contributed to the variation within some lineages and eventually resulted in further speciation at the homoploid level. Apparently, cleistogamy has played an important role in the stabilisation of hybrid derivatives. Furthermore, secondary hybridisation among some tetraploid lineages has apparently resulted in new reticulations at the octoploid level. At present, subsection *Viola* appears as a dynamic polyploid complex, where extant species can be viewed as temporarily stabilised genotypes. The current subdivision into two series, *Flagellatae* and *Eflagellatae*, probably does not reflect true phylogenetic relationships within this group.

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References

Anonymous (1996) Flora of China checklist and distribution maps (version 07. Sep. 1998). URL http://mobot.mobot.org/Pick/Search/index/fact.html

Arnold M. L. (1997) Natural hybridization and evolution (Oxford series in ecology and evolution). Oxford University Press, Oxford.

Arnold M. L., Bulger M. R., Bruke J. M., Hempel A. L., Williams J. H. (1999) Natural hybridization: How long can you go and still be important? Ecology 80: 371–381.

Asins M. J., Breto M. P., Cambra M., Carbonell E. A. (1993) Salt tolerance in *Lycopersicon* species: I. Character definition and changes in gene expression. Theor. Appl. Genet. 86: 737–743.

Asins M. J., Herrero R., Navarro L. (1995) Factors affecting *Citrus* tree isozyme-gene expression. Theor. Appl. Genet. 90: 892–898.

Ballard H. E. Jr., Sytsma K. J., Kowal R. R. (1999) Shrinking the violets: Phylogenetic relationships of infrageneric groups in *Viola* (Violaceae) based in internal transcribed spacer DNA sequences. Syst. Bot. 23: 439–458.

Banasinska A., Kuta E. (1996) Allogamy in *Viola odorata* L. Acta Biol. Cracov. Ser. Bot. 38: 41–53.

Barrett S. C. H., Kohn J. R. (1991) Genetic and evolutionary consequences of small population size in plants. In: Falk D. A., Holsinger K. E. (eds.) Genetics and conservation of rare plants. Oxford University Press, New York, pp. 3–27.

Beattie A. J. (1969a) The floral biology of three species of *Viola*. New Phytol. 68: 1187–1201.

Beattie A. J. (1969b) The pollination ecology of *Viola* 1. Pollen containts in stigmatic cavities. Watsonia 7: 142–156.

Beattie A. J. (1971) Pollination mechanisms in *Viola*. New Phytol. 70: 343–360.

Beattie A. J. (1972) The pollination ecology of *Viola* 2. Pollen loads of insect-visitors. Watsonia 9: 13–25.

Beattie A. J. (1974) Floral evolution in *Viola*. Ann. Missouri Bot. Gard. 61: 781–793.

Becker W. (1903) *Viola sepincola* Jord. 1849 = *Viola Beraudii* Bor. 1857 = *Viola Austriaca* A. & J. Kerner 1872 = *Viola cyanea* Čel. 1872. Allg. Bot. Z. 9: 114–118.

Becker W. (1908) Beiträge zur Violenflora Asiens. Bull. Herb. Boissier, ser. 2: 739–744.

Becker W. (1910) Violae Europaeae. Verlag von C. Heinrich, Dresden-N.

Becker W. (1918) Violae Asiaticae et Australenses III. Beih. Bot. Ctrlbl. 36: 15–59.

Becker W. (1925) In: Engler A., Prantl K. (eds.) Die natürlichen Pfanzenfamilien 21: 363–376.

Bergdolt E. (1932) Morphologische und physiologische Untersuchungen über *Viola*. Bot. Abh. 20: 8–120.

Bethke A. (1882) Über die Bastarde der Veilchen-Arten. Inaugural-Dissertation zur Erlangung der Doctorwürde von der philosophischen Facultät der Albertus-Universität zu Königsberg i. Pr.: 1–20.

Candolle A. P. De (1824) *Viola serpens* Wall. Candolle, prodr. 1: 296.

Chodat L. (1924) Contributions à la géo-botanique de Majorque. Genève: Thèse No. 734.

Clausen J. (1929) Chromosome number and the relationship of some North American species of *Viola*. Ann. Bot. 43: 741–764.

Clausen J. (1962) Stages in the evolution of plant species. Hafner Publishing Company, New York.

Crawford D. J. (1989) Enzyme electrophoresis and plant systematics. In: Soltis D. E., Soltis P. S. (eds.) Isozymes in plant biology. (Advances in plant sciences, series 4) pp. 146–164.

Culley T. M. (submitted) Inbreeding depression in *Viola canadensis* (Violaceae), a species with chasmogamous and cleistogamous flowers (preliminary title).

Culley T. M., Wolfe A. D. (2000) Population genetic structure of the cleistogamous plant species, *Viola pubescens*, as indicated by isozyme and ISSR molecular markers. Mol. Ecol. (in press).

Culver D. C., Beattie A. J. (1980) The fate of *Viola* seeds dispersed by ants. Am. J. Bot. 67: 710–714.

Demesure B., Comps B., Petit J. R. (1996) Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. Evolution 50: 2515–2520.

Dizerbo A. H. (1967) Observations sur *Viola odorata* L., *Viola hirta* L. et leur hybride *Viola permixta* Jord. dans le Massif Armoricain I-II. Bull. Soc. Sci. Bretagne 42: 113–124 & 215–223.

Dizerbo A. H. (1968) Observations sur *Viola odorata* L., *Viola hirta* L. et leur hybride *Viola permixta* Jord. dans le Massif Armoricain III-IV. Bull. Soc. Sci. Bretagne 43: 71–79 & 225–236.

Dodd J. D., Gershoy A. (1943) Experiments in the grafting of species in the genus *Viola*. Bull. Torrey Bot. Club 70: 91.

Erdner E. (1907) Sind die Veilchenbastarde fruchtbar oder nicht? Allg. Bot. Zeitschr. 7/8: 117–118.

Fægri K. (1996) Introduction. In: Fægri K., Danielsen A. (eds.) Maps of distribution of Norwegian vascular plants, Vol. 3. The southeastern element. Fagbokforlaget, Bergen, pp. 9–16.

Fukarek F. (1995) Florenentwicklung im Eiszeitalter. In: Fukarek F. (ed.) Urania Pflanzenreich 4. Vegetation, Urania-Verlag, Leipzig, pp. 61–67.

Gams H. (1925) Violaceae. In: Hegi G. (ed.) Illustrierte Flora von Mittel-Europa, Vol. 5. J. F. Lehmanns Verlag, München, pp. 585–657.

Gershoy A. (1934) Studies in North American violets III. Chromosome numbers and species characters. Vermont Agric. Sta. Bull. 367: 1–91.

Gerstlauer L. (1943) Vorschläge zur Systematik der einheimischen Veilchen. Ber. Bayer. Bot. Ges. 26: 12–55.

Gil-ad N. L. (1997) Systematics of *Viola* subsection *Boreali-Americanae*. Boissiera 53: 1–130.

Gower J. C. (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrica 53: 325–338.

Grime J. P., Hodgson J. G., Hunt R. (1990) The abridged plant ecology. Unwin Hyman, London.

Grintescu G., Gusuleac M., Nyárády E. I. (1955) *Viola* L. In: Savulescu T. (ed.) Flora Republicii Populare Romîne, Vol. 2. Editura Academiei Republicii Populare Romîne.

Haesler I. (1982) Lectotypisierung der Arten *Viola hirta* L. und *Viola odorata* L. Mitt. Bot. München 18: 289–96.

Heilborn O. (1926) Bidrag til Violaceernas cytologi. Sven. Bot. Tidskr. 20: 414–419.

Hess H. E., Landolt E., Hirzel R. (1970) Flora der Schweiz. Birkhäuser, Basel.

Hiemeyer F. (1992) Über einheimische Veilchen und ihre Kreuzungen im Mittelschwäbischen Raum – Beobachtungen und Erkenntnisse. Ber. Bayer. Bot. Ges. 63: 81–102.

Hultén E., Fries M. (1986) Atlas of North European vascular plants, Vol. 2. Koeltz Scientific Books, Königstein.

Kephart S. (1990) Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. Am. J. Bot. 77: 693–712.

Kim K. S., Sun B. Y., Whang S. S., Chung G. H. (1991) Comparative morphology of the *Viola albida* complex. Korean J. Bot. 34: 229–238.

Kosonen L., Kaipiainen H., Kemppainen E. (1996) Suomen uhanalaiset lajit: Mäkiorvokki (*Viola collina*). Suomen ympäristö 75: 1–37.

Marcussen T., Borgen L., Nordal I. (submitted) Viola hirta and its relatives in Norway.

Marcussen T., Nordal I. (1998) *Viola suavis*, a new species in the Nordic flora, with analyses of the relation to other species in the subsection *Viola* (Violaceae). Nord. J. Bot. 18: 221–237.

Melchior H. (1939) Ein neues Veilchen aus SW.-Anatolien und die Phylogenie der Sprossentwicklung innerhalb der Sektion *Nomimium*. Feddes Repert. 46: 39–42.

Meusel H. (1978) Vergleichende Chorologie der Zentraleuropäischen Flora, Vol. 2. VEB Gustav Fischer Verlag, Jena.

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

Moore D. M. (1959) Population studies on *Viola lactea* Sm. and its wild hybrids. Evolution 13: 318–332.

Morden C. W., Doebley J., Schertz K. F. (1987) A manual of techniques for starch gel electrophoresis of *Sorghum* isozymes. Texas: The Texas Agric. Exp. Stn. The Texas A and M Univ. System, College Station.

Mowrey B. D., Werner D. J. (1990) Developmental specific isozyme expression in peach. Hortscience 25: 219–222.

Muñoz Garmendia F., Montserrat P., Laínz M., Aldasoro J. J. (1993) *Viola* L. In: Castroviejo S., Aedo C., Cirujano S., Laínz M., Montserrat P., Morales R., Muñoz Garmendia F., Navarro C., Paiva J., Soriano C. (eds.) Flora Iberica, Vol. 3. Real Jardín Botánico, C.S.I.C., Madrid.

Neuffer B., Auge H., Mesch H., Amarell U., Brandl R. (1999) Spread of violets in polluted pine forests: Morphological and molecular evidence for the ecological importance of interspecific hybridization. Mol. Ecol. 8: 365–377.

Nordal I. (1996) *Viola collina*, *V. hirta* and *V. odorata*. In: Fægri K., Danielsen A. (eds.) Maps of distribution of Norwegian vascular plants, Vol. 3. The southeastern element. Fagbokforlaget, Bergen, pp. 115–116.

Nordal I., Jonsell B. (1998) A phylogeographic analysis of *Viola rupestris* – three postglacial immigration routes to the Nordic area? Bot. J. Linn. Soc. 128: 105–122.

Okamoto M., Okada H., Ueda K. (1993) Morphology and chromosome number of *Viola pilosa*, and its systematic position. Taxon 42: 781–787.

Petit C., Bretagnolle F., Felber F. (1999) Evolutionary consequences of diploid-polyploid hybrid zones in wild species. Trends Ecol. Evol. 14: 306–311.

Polunin O., Walters M. (1985) A guide to the vegetation of Britain and Europe. Oxford University Press, Oxford.

Redbo-Torstensson P., Berg H. (1995) Seasonal cleistogamy: a conditional strategy to provide reproductive assurance. Acta Bot. Neerl. 44: 247–256.

Rieseberg L. H., Ellstrand N. C. (1993) What can molecular and morphological markers tell us about plant hybridization? Crit. Rev. Pl. Sc. 12: 213–241.

Schmidt A. (1961) Zytotaxonomische Untersuchungen an europäischen *Viola*-Arten der Sektion *Nomimium*. Österr. Bot. Zeitschr. 108: 20–88.

Schmidt A. (1962) Eine neue Grundzahl in der Gattung *Viola*. Zytotaxonomische Untersuchungen an *Viola parvula* Tin. und *Viola occulta* Lehm. Ber. Deutsch. Bot. Ges. 75: 78–84.

Schmidt A. (1964) Zur systematischen Stellung von *Viola chelmea* Boiss. Heldr. ssp. *chelmea* und *V. delphinantha* Boiss. Ber. Deutsch. Bot. Ges. 77: 256–261.

Schnarf K. (1922) Zur Samenentwicklung einiger *Viola*-Bastarde. Österr. Bot. Zeitschr. 71: 190–199.

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

Sokal R. R., Michener C. D. (1958) A statistical method for evaluating systematic relationships. Univ. of Kansas Sc. Bull. 38: 1409–1438.

Soltis D. E., Soltis P. S. (1989) Polyploidy, breeding system, and genetic differentiation in homosporous pteridophytes. In: Soltis D. E., Soltis P. S. (eds.) Isozymes in plant biology. (Advances in plant sciences, series 4), pp. 241–258.

Soltis D. E., Soltis P. S. (1993) Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. 12: 243–273.

Soltis D. E., Soltis P. S. (1999) Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. 14: 348–352.

Strid A. (1986) Mountain flora of Greece. Cambridge University Press, Cambridge, p. 616.

Taberlet P. (1998) Biodiversity at the intraspecific level: The comparative phylogeographic approach. J. Biotech. 64: 91–100.

Taberlet P., Fumagalli L., Cosson A. M., Wust S., Andjean F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7: 453–464.

Tchourina O. (1909) Note sur le *Viola Jaubertiana* Marès [sic]. Bull. Soc. Bot. Genève (Serie 2) 1: 204–207.

Valentine D. H. (1962) Variation and evolution in the genus *Viola*. Preslia 34: 190–206.

Valentine D. H. (1975) *Viola* L. In: Stace C. A. (ed.) Hybridization and the Flora of the British Isles. Academic Press, London, pp. 154–163.

Valentine D. H., Merxmüller H., Schmidt A. (1968) *Viola* L. In: Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M., Webb D. A. (eds.) Flora Europaea, Vol. 2. Cambridge University Press, Cambridge, pp. 270–282.

Wendel J. F. (1999) Genome evolution in polyploids. Pl. Mol. Biol. (in press).

Wendel J. F., Weeden N. F. (1989) Visualization and interpretation of plant isozymes. In: Soltis D. E., Soltis P. S. (eds.) Isozymes in plant biology. (Advances in plant sciences series 4), pp. 5–45.

Werth C. R. (1989) The use of isozyme data for inferring ancestry of polyploid pteridophytes. Biochem. Syst. Ecol. 17: 177–130.

Yuzepchuk S. V. (1949) *Viola* L. In: Shishkin B. K. (ed.) Flora of the U.S.S.R., Vol. 15. Israel: Israel Program for Scientific Translations (1974).

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