Plant Syst. Evol. 223: 155–171 (2000)

Plant Systematics and Evolution

The evolution of pollen heteromorphism in *Viola*: A phylogenetic approach

S. Nadot[1], **H. E. Ballard Jr**[2], **J. B. Creach**[1], and **I. Dajoz**[3]

[1]Laboratoire Evolution et Systématique, Université Paris-Sud, Orsay, France
[2]Department of Environmental and Plant Biology, Porter Hall, Ohio University, Athens, OH, USA
[3]Laboratoire d'Ecologie, Ecole Normale Supérieure, Paris, France

Received October 4, 1999
Accepted January 25, 2000

Abstract. Pollen heteromorphism, defined here as the production within all flowers of a plant of several pollen morphs differing in aperture number, is common in angiosperms. We have focused on the evolution of pollen heteromorphism in the genus *Viola,* in which about 1/3 of the species are pollen-heteromorphic. We have studied the distribution of pollen heteromorphism in the genus *Viola* using a molecular phylogeny based on ITS sequence data. We show that pollen heteromorphism has evolved independently at least six times in *Viola.* A comparative analysis shows that the occurrence of pollen heteromorphism is correlated with sporophytic polyploidy in all sections of the genus apart from section *Melanium.* This section differs from all other sections on several aspects such as flower morphology, absence of cleistogamous flowers, and a high proportion of heteromorphic species. We discuss the possible adaptiveness of pollen heteromorphism in this section.

Key words: Pollen heteromorphism, *Viola* (Violaceae), phylogeny, comparative analysis, sporophytic ploidy level.

Pollen heteromorphism is defined as the production by the same plant of several pollen grain morphs that differ in aperture number (Till-Bottraud et al. 1995). This phenomenon occurs in over 30% of angiosperm species (Mignot et al. 1994). To study the evolution of pollen heteromorphism, we have focused on the genus *Viola,* in which pollen heteromorphism is common: among the 525 species of the genus (Ballard 1996), about 1/3 of analyzed species are pollen-heteromorphic (see Appendix 1). Pollen morphs with three to six apertures are found in the genus, and this range of variation can even be present within the same plant (Dajoz et al. 1993).

The genus *Viola* is subdivided into several sections. The section *Melanium* represents the so-called Pansies and all the other sections correspond to the commonly named Violets. Pansies and Violets are distinguished on the basis of differences in flower morphology (Tutin et al. 1968): the two lateral petals are directed downwards in Violets and upwards in Pansies. Several other differences separate Pansies from Violets, such as (i) geographic distribution: Pansies are found only in Europe and western Asia, whereas Violets are almost world-wide (Clausen 1929); (ii) the occurrence of pollen heteromorphism: preliminary observations carried out on 28 European species of *Viola* showed that 81% of the species of Pansies were pollen-heteromorphic, whereas only 42% of the Violets were

heteromorphic (Dajoz 1999); (iii) there is much evidence from the literature (and from our own field observations) that other traits of flower morphology (corolla size, length of the nectar spur) and pollination ecology are very different between the two groups (Beattie 1971, 1974; Herrera 1990, 1993; Ballard 1996). Pansies produce only open (chasmogamous) flowers (Knuth 1908, Herrera 1993), whereas Violets typically produce both showy, attractive, chasmogamous flowers and highly reduced, closed, self-fertilizing cleistogamous flowers (Beattie 1969, Grime et al. 1986).

Why should plants produce different pollen morphs? Because pollen tube germination occurs through the aperture, we may reasonably hypothesize that changes in aperture number could influence some parameters of pollen grain fitness. In four species of *Viola*, it has been observed that the different pollen morphs differ with respect to two fitness components: aperture number is positively correlated with germination speed, but negatively with life-expectancy; (Dajoz et al. 1993, I. Skogsmyr, unpubl. results, Till-Bottraud et al. 1999). A game theory model established by Till-Bottraud et al. (1994) predicts that pollen heteromorphism is an Evolutionary Stable Strategy (ESS) if there is a trade-off between germination speed and life-expectancy of the pollen grain, and if pollination conditions vary in an unpredictable way. Natural selection should favour many-aperturate quickly germinating pollen grains when pollinators are abundant, and longer-lived pollen grains with fewer apertures when pollinators are scarce. Therefore, we may hypothesize that aperture number varies with several traits of pollination ecology and pollen physiology.

Here we study the distribution of pollen heteromorphism in the genus *Viola* in order to understand which selective pressures may act on its maintenance, using the molecular phylogeny based on ITS sequence data of Ballard (1996). The use of phylogenetic analyses to address evolutionary questions has proven useful when competing hypotheses are available concerning character evolution (De Fraipont et al. 1996, Kohn et al. 1996, Schoen et al. 1997, Rolland et al. 1998). A phylogenetic approach is especially suitable for studying the evolution of pollen heteromorphism, because it arises frequently in angiosperms in general (Erdtman 1966, Mignot et al. 1994) and in the genus *Viola* in particular (Dajoz 1999). Most of all, the respective roles of ploidy level variation of the sporophyte and of particular selective pressures that may render pollen heteromorphism adaptive need to be clarified in order to better understand the evolution of pollen aperture number. Since pollen heteromorphism is often correlated with variation in the sporophytic ploidy level (Bronckers 1963, Erdtman 1966) we investigated sporophyte ploidy level variation.

Consequently, our first aim in this paper is to study the distribution of pollen heteromorphism within the genus. This is done by comparing the proportion of pollen-heteromorphic species among Pansies and the different groups of Violets, using a world-wide survey carried out on 158 species of *Viola*. Second, using a molecular phylogeny of *Viola*, we trace the evolution of pollen heteromorphism in order to identify the ancestral condition of pollen morphology in the genus. We then use the comparative method to test whether the occurrence of pollen heteromorphism is related to variation in ploidy level of the sporophyte. Finally, we examine pollination ecology and floral biology as potential selective pressures on pollen aperture number, using data from the literature in conjunction with our own observations on variation in reproductive and floral traits of several *Viola* species.

Material and methods

Overview of the genus. The species of *Viola* are found mostly in the temperate but also in the tropical zones of both hemispheres (Clausen 1929) and in a wide array of environmental conditions, from lowland cultivated fields, meadows and forests to alpine habitats (Tutin et al. 1968). *Viola*

species exhibit a range of growth forms, life cycles and breeding systems. The genus includes small shrubs or herbs, plants with annual and perennial life-cycles (Tutin et al. 1968), as well as selfing and outcrossing species (Beattie 1969, Grime et al. 1986, Herrera 1993). All outcrossing species are insect pollinated (Knuth 1908).

Preparation of pollen slides. Pollen was sampled from 158 species, using fresh or herbarium specimens. Species sampled, type of plant material used and herbarium supplying the specimens are indicated in Appendix 1. For each species, pollen was sampled from one to five different populations and one to two plants per population, whenever possible, and one randomly chosen flower per plant. Pollen grains from each plant were collected, mounted in glycerine jelly stained with fuchsin and examined with a light microscope. Number of apertures was recorded in at least 40 pollen grains per slide. For each species sampled, sporophyte chromosome number and ploidy level were taken from the literature (Goldblatt and Davidse 1981, 1984, 1985; Goldblatt 1988; Goldblatt and Johnson 1990; Ballard 1996).

Statistical analyses. For each plant from which enough pollen was available, we calculated the mean and the variance for aperture number of pollen grains per plant. The variance in aperture number (s^2_{ap}) was then averaged for each species, when data from several individuals were available (we checked that interspecific variance was higher than intraspecific variance). All species with mean variance above 0.0475 were considered pollen-heteromorphic. We followed the definition of Mignot et al. (1994) according to which a plant is considered heteromorphic when the most abundant pollen morph represents less than 95% of all morphs. This proportion corresponds to the threshold value of 0.0475 for the variance.

Statistical tests were carried out using SAS (SAS 1989). We compared s^2_{ap} among different subgroups of the genus, using nonparametric test because s^2_{ap} was not normally distributed, then tested whether s^2_{ap} was significantly different for Violets *s.l.* versus Pansies (Mann-Whitney two-sample test). Moreover, we examined different monophyletic groups of Violets versus Pansies, using a Kruskal-Wallis test followed by nonparametric multiple comparisons of means.

The phylogeny. Only two phylogenies of *Viola* are available in the literature. The older, based on variation of chromosome numbers, considers the relationship among some European species on the one hand (Clausen 1927) and some North American species on the other (Clausen 1929). We employ here a recent molecular phylogeny of the genus, based on ITS (nuclear ribosomal internal transcribed spacer) sequence data (Ballard 1996), including more than 50 species representing most of the morphologically distinct subgroups currently recognized in the genus. The two states (presence/absence) of the character pollen heteromorphism were mapped on both the Maximum Parsimony and the Maximum Likelihood trees, using MacClade version 3.01 (Maddison and Maddison 1992), which we also used for testing different character weighting schemes (Kohn et al. 1996, Schoen et al. 1997).

Comparative analysis. Different methods were used to test for a relationship between pollen heteromorphism and ploidy level: Sillén-Tullberg's method for testing the contingency of states in two characters (Sillén-Tullberg 1993, Lindefors 1997), and Pagel's (1994, 1997) general method of comparative analysis for discrete variables. We also used the concentrated-changes test of MacClade 3.01 (Maddison and Maddison 1992), which tests the association of changes in two binary characters. All methods were applied to Ballard's (1996) molecular phylogeny, from which we removed all species for which data were not available.

Results

Distribution of pollen heteromorphism in Pansies and Violets. Pansies (i.e., section *Melanium*) and Violets (all other sections) differ in the proportion of species exhibiting pollen heteromorphism: 74% versus 17% respectively. The proportions are not noticeably altered when the main monophyletic sections of Violets are considered separately (Table 1). Pansies and Violets differ also in the range of pollen types: only morphs with three and four apertures are found in Violets, whereas additional morphs with five and six apertures occur in Pansies. Moreover, the most abundant pollen morph differs between both groups: 3-aperturate pollen grains are widespread in Violets versus 4-aperturate pollen grains in Pansies.

Table 1. Proportion of heteromorphic species in Pansies and in different monophyletic sections of Violets, and number of apertures of the pollen morphs encountered in each group ([a]most abundant morph; [b]next most abundant morph)

	Chamaemelanium	*Plagiostigma*	*Viola* + *Nosphinium*	*Melanium*
Number of species analyzed	14	34	45	47
Proportion of heteromorphic species	14%	15%	24%	74%
Pollen morphs (number of apertures)	3[a], 4[b]	3[a], 4[b]	3[a], 4[b]	3, 4[a], 5[b], 6

In heteromorphic species, the relative proportion of the different morphs can vary extensively. Figure 1 shows the frequency distribution of variance in mean aperture number among species. The distribution for Violets is very left-skewed, whereas values for Pansies are more evenly distributed. The mean of s^2_{ap} differs significantly between Violets and Pansies (Violets: $s^2_{ap} = 0.027$, Pansies: $s^2_{ap} = 0.094$; $Z = 6.28$, d.f. = 1, $P = 0.0001$, Mann-Whitney test). The variance in mean aperture number is significantly higher in section *Melanium* than in the main monophyletic groups of Violets taken separately (Table 2).

Evolution of pollen heteromorphism in *Viola*. The molecular phylogeny (Fig. 2) obtained by Ballard (1996), although representing only 10% of the total number of species accepted as distinct taxa in the genus, clearly shows that the so-called Violets are actually paraphyletic, and that some of the sections are not monophyletic (e.g., *Chamaemelanium*, *Plagiostigma*). Pansies are represented by only three species (the whole section includes about

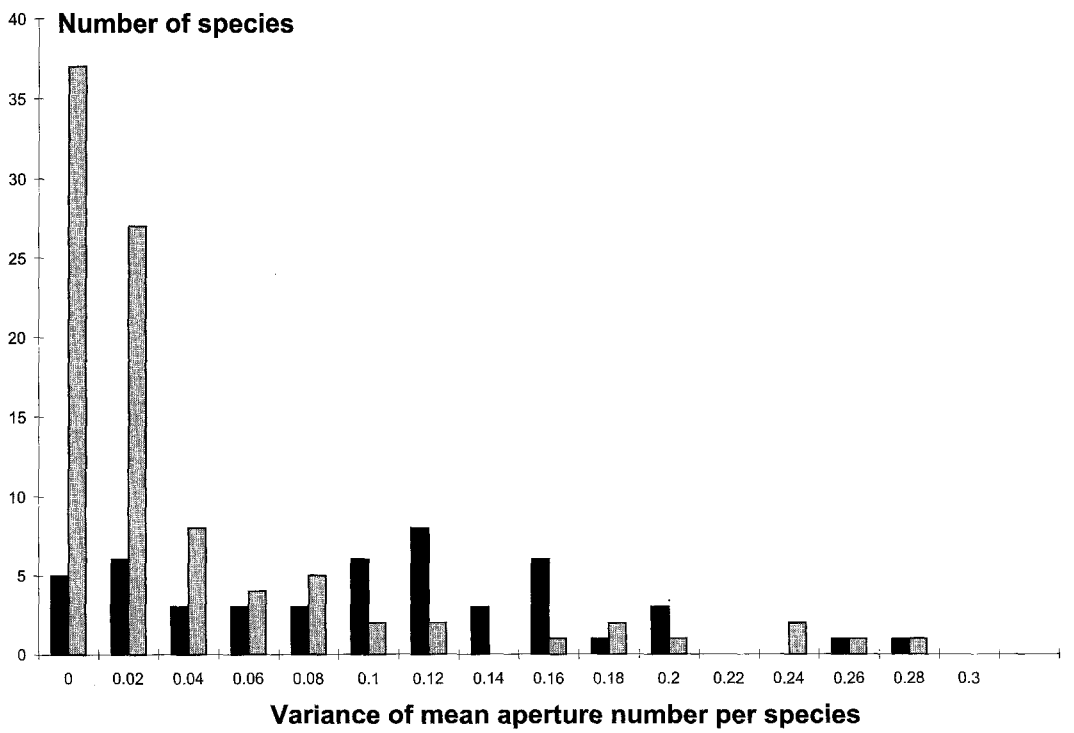


Fig. 1. Frequency distribution of the 158 species studies according to their variance in mean aperture number. Grey bars: Violets; black bars: Pansies

Table 2. Comparison of the variance in mean aperture number among *Melanium* and the main sections of Violets. We considered four monophyletic groups of comparable sizes: *Chamaemelanium 1*, *Melanium*, *Plagiostigma 1+2* and *Viola-Nosphinium*. Chi square approximation of Kruskal-Wallis test: $\chi^2 = 32.99$, d.f. = 3, P = 0.0001. Shared lower case letters indicate groups that did not differ by a posteriori Tukey-type nonparametric test

Section	*Chamaemelanium*	*Plagiostigma*	*Viola-Nosphinium*	*Melanium*
Sample size	14	31	47	47
Mean	0.023	0.025	0.037	0.097
Tukey grouping	a	a	a	b

a hundred species, Tutin et al. 1968). They appear monophyletic and derived from Violets. The addition/removal of taxa slightly changes the topology (Ballard et al. 1999), but the pansies clade always comes out as a sister group to the *Viola-Nosphinium* clade and derived from violets.

Mapping the character pollen heteromorphism onto the phylogeny (Fig. 2) shows that 3-aperturate non-heteromorphic pollen is the ancestral condition of pollen morphology in *Viola*. From this ancestral condition, pollen heteromorphism evolved at least six times independently. There are at least three different origins in the combined clade *Viola-Nosphinium* (two species are heteromorphic in the clade *Nosphinium*, but due to missing data it is difficult to decide between one or two origins, although MacClade indicates two origins without ambiguity) and one origin in each section *Chamaemelanium* and *Plagiostigma*. All three species representing the section *Melanium* are heteromorphic, so pollen heteromorphism evolved at least once in this section. We tested the influence of the tree topology on the evolution of the character by mapping it on the Maximum Likelihood tree obtained with the same data set (Ballard 1996), which presents a slightly different topology. The results were not affected (Fig. 3a).

To make sure that considering the presence of heteromorphism as derived within *Viola* is the most parsimonious hypothesis, we tried different assumptions for the evolution of this character. When we assumed that the presence of heteromorphism was the ancestral state, we found that it involved one more step than considering heteromorphism as derived (Fig. 3b). We also treated pollen heteromorphism as a weighted character in two different ways, by weighting the acquisition of heteromorphism 2:1 (Fig. 3c and d), or 1:2 (trees are identical to those shown on Fig. 3a and b). In both cases, we found that it was more parsimonious to assume that heteromorphism is derived.

Pollen heteromorphism and ploidy level of the sporophyte. Figure 4 presents the two phylogenies used to examine the relationship between pollen heteromorphism and ploidy level of the sporophyte. They have the same topology as the tree shown in Fig. 2, but species for which data are missing have been omitted. The states of the independent character (ploidy level of the sporophyte) and of the dependent character (presence/absence of heteromorphism) have been indicated on the tree. The difference between trees A and B concerns the section *Melanium*, which has been removed in tree B. Indeed, the proportion of heteromorphic species that are diploid is much higher in *Melanium* than in any other section of the genus (75% versus no more than 12% – percentages calculated from appendix), suggesting no direct link between ploidy level and pollen heteromorphism. It makes it questionable to consider *Melanium* together with the rest of the genus when examining a possible correlation between polyploidization and pollen heteromorphism.

Table 3 summarizes the results obtained using Sillén-Tullberg's method. For both trees

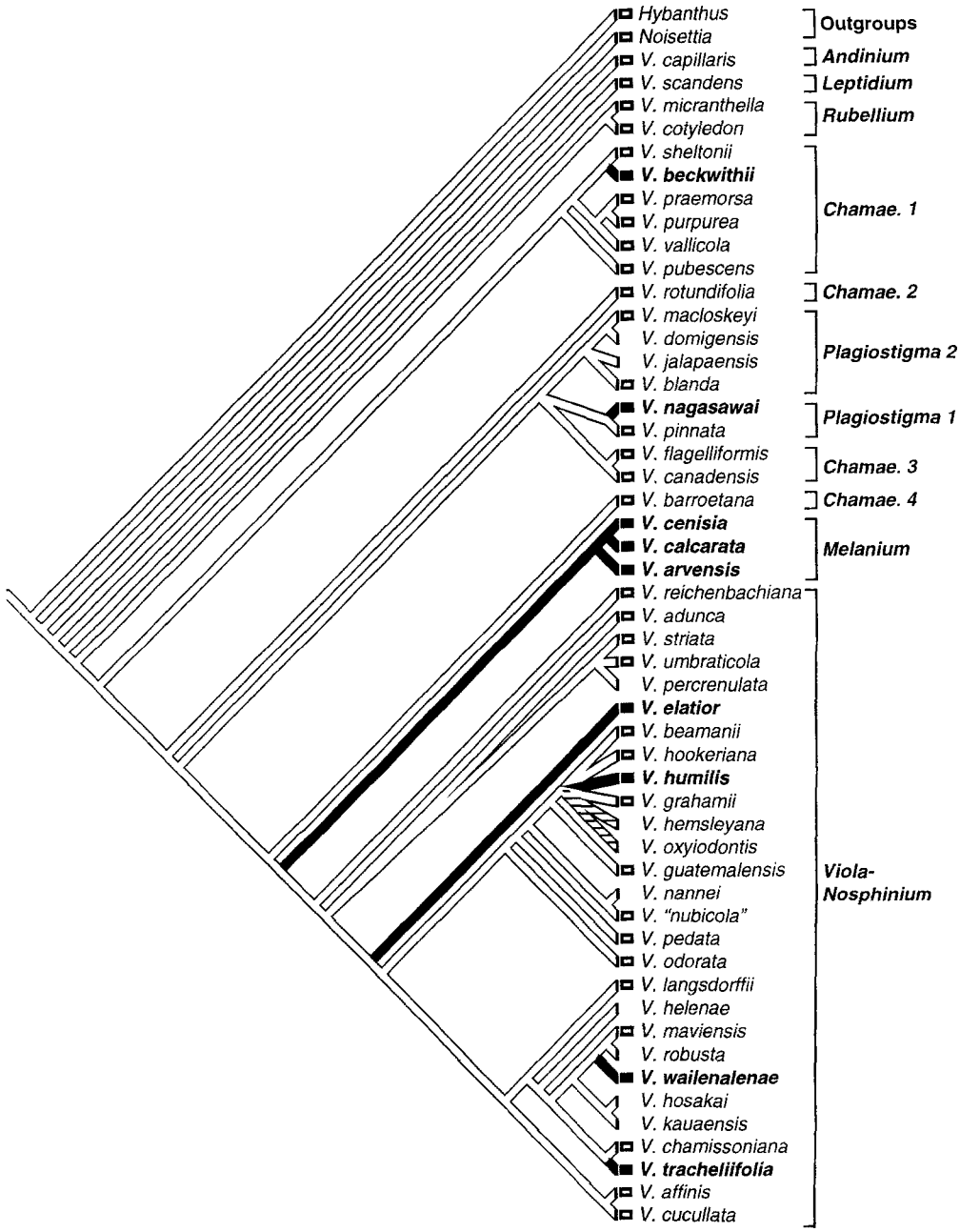


Fig. 2. Molecular phylogeny of *Viola* based on ITS sequence data (Ballard 1996). This tree is the consensus of the 60 most parsimonious trees (1279 steps) from maximum parsimony analysis. The character pollen heteromorphism has been mapped on this tree with the help of MacClade version 3.01. Black branches lead to species showing the presence of heteromorphism. Black boxes indicate heteromorphic species, white boxes non heteromorphic species, and species with no box are those for which pollen data is missing. The systematic section according to Ballard is indicated on the right. *Chamae.* = *Chamaemelanium*

A and B, the contingency test shows that the acquisition of heteromorphism is contingent upon polyploidization events in the genus. Likewise, MacClade's concentrated-changes test detects a significant correlation between the two characters in both trees. Pagel's

omnibus test detects a significant correlation between polyploidy and the presence of heteromorphism in tree A (Likelihood Ratio = −11.88; tested against χ^2, d.f. = 4, $P < 0.05$) but not in tree B (Likelihood Ratio = 6.63; tested against χ^2, d.f. = 4, NS).

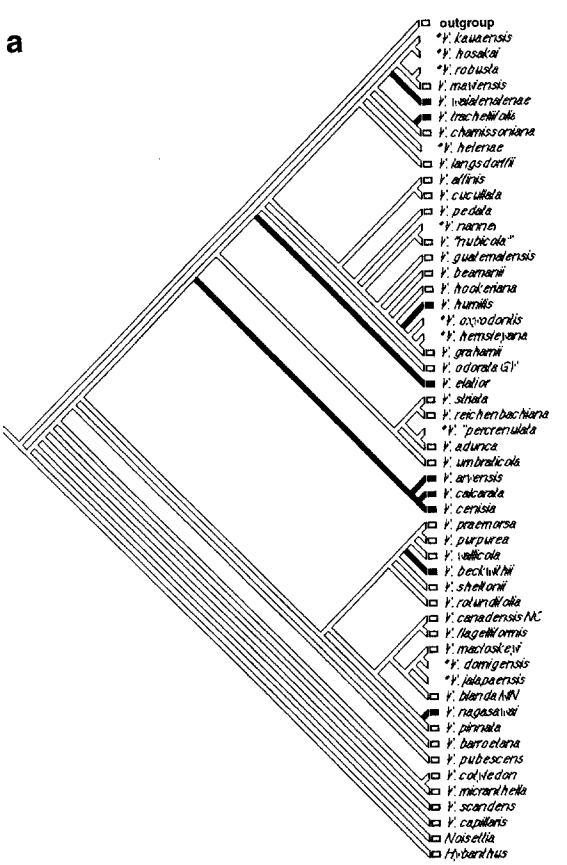
Discussion

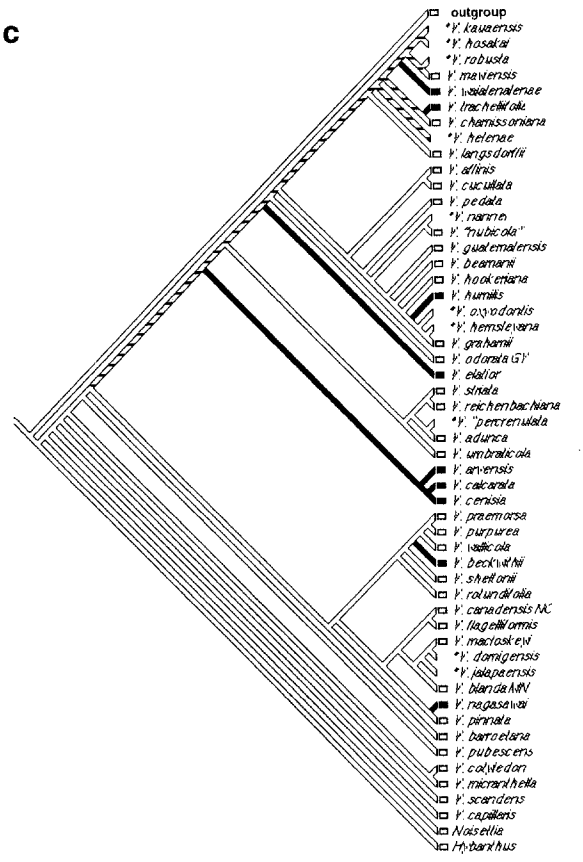
Pansies versus Violets. The section *Melanium* (Pansies) stands out from the rest of the genus, as shown by all traits studied concerning pollen heteromorphism.

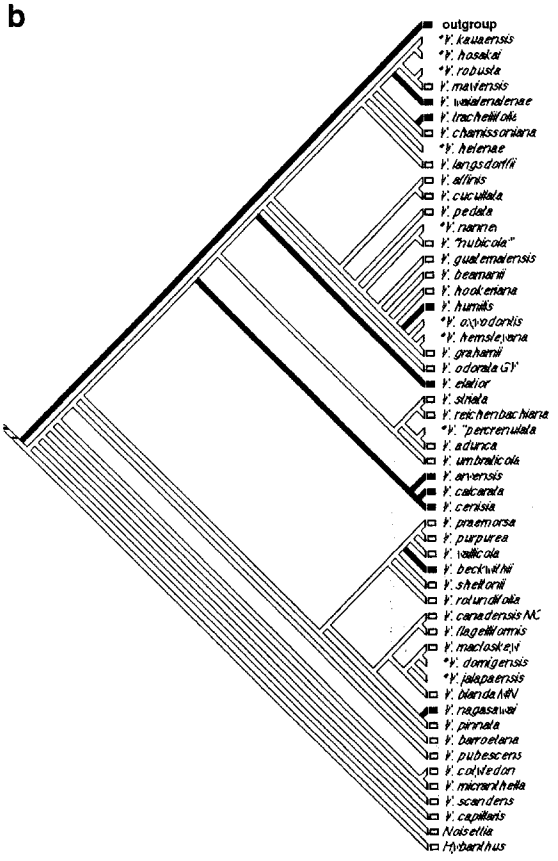
Our data mapped on the phylogeny show that pollen heteromorphism has arisen at least six times independently: at least once in *Melanium*, and several times in the rest of the genus. As shown by the analyses with MacClade, the 3-aperturate pollen grain morph is the ancestral condition in *Viola*. Therefore, pollen heteromorphism and the occurrence of 4-, 5- and 6-aperturate pollen grains are considered derived conditions. This is in agreement with data from the angiosperm fossil record, which shows that the earliest Eudicotyledons had triaperturate pollen grains (Crane et al. 1995). The fossil record also gives evidence for an increase in mean aperture number over evolutionary time (Walker and Doyle 1975, Doyle and Hotton 1991). Furthermore, a comparison between taxa with ancestral and derived traits in angiosperms shows that there has been an increase in pollen germination speed (Mulcahy 1979, Hoekstra 1983). It means that there is a potential variability of pollen morphology and physiology in angiosperms, which has been selected towards one direction (increase in aperture number and germination speed). This suggests that selective forces can act on the male gametophyte, therefore influencing its fitness.

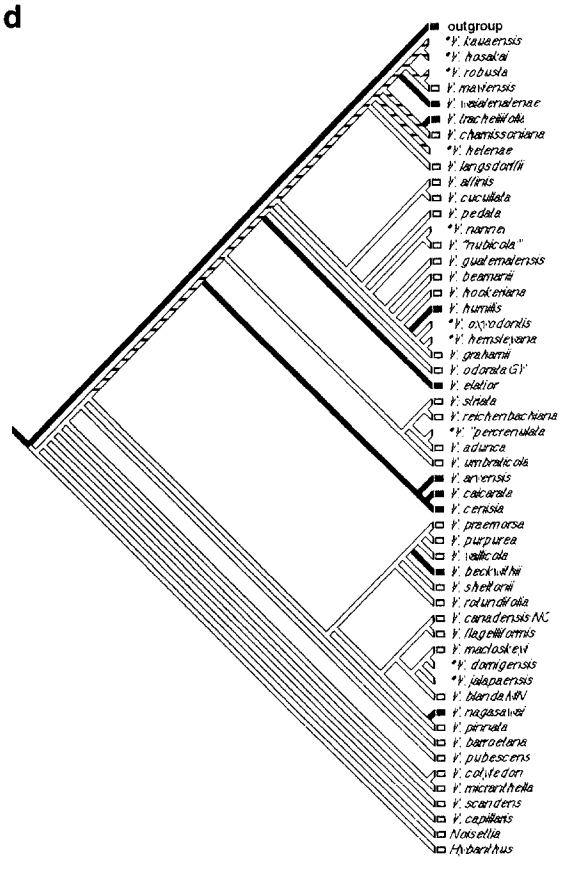
The origin of pollen heteromorphism. The comparative analysis was performed on two different trees, including or not section *Melanium* (Fig. 4). Pagel's method (omnibus test) detected a significant correlation between ploidy level and pollen heteromorphism only in tree B, when *Melanium* was omitted. This shows that the omnibus test is sensitive to the addition of new data. The most likely explanation for this is that there are few heteromorphic species represented in the phylogeny, and the inclusion of section *Melanium* adds two heteromorphic but diploid species, thus lowering the correlation between the two characters studied. When using Sillén-Tullberg's method, the correlation remains significant when *Melanium* is included, even though the significance level is lower than the one obtained with tree A. Overall, these analyses show that in the genus *Viola* (except section *Melanium*), the occurrence of pollen heteromorphism is significantly related to ploidy level, as shown by the comparative analysis. This is in agreement with experimental data concerning the influence of induced polyploidy on pollen morphology: in *Arabidopsis* (Bronckers 1963), *Salvia* (Erdtman 1966) and *Petunia* (Mignot et al. 1994), diploids produce only 3-aperturate pollen grains, whereas polyploids are pollen-heteromorphic. This gives evidence that, in these species, pollen heteromorphism is a direct consequence of polyploidy. It also justifies our decision of considering only two states for the ploidy level (diploidy/polyploidy) without taking into account the different ploidy levels of the polyploids studied.

Theoretical predictions and observations are available concerning the mechanisms that could be involved during meiosis in the morphogenesis of pollen grains. Observations show that pollen aperture number is determined just after the completion of meiosis, when the microspores are still grouped in tetrads (Stainier et al. 1967). In tobacco, the structure of the meiotic spindle is dependent upon the ploidy level of the mother cell, and this structure will in turn influence the relationships among the four microspores, leading to different pollen morphs with different aperture numbers within the same tetrad (Ressayre et al. 1998). Observations on the structure of the meiotic spindle in heteromorphic species show that its different configurations lead to the production of different pollen morphs (Ressayre, pers. comm.).


a
outgroup
*V. kawaensis
*V. hosakai
*V. robusta
V. mayensis
V. walalealensis
V. trachelifolia
V. chamissoniana
*V. helenae
V. langsdorffii
V. affinis
V. cuculiata
V. pedata
*V. nannen
V. "nubicola"
V. gualemalensis
V. beamanii
V. hookeriana
V. humilis
*V. oxyodontis
*V. hemsleyana
V. grahamii
V. odorata GV
V. elatior
V. striata
V. reichenbachiana
*V. "percrenulata
V. adunca
V. umbraticola
V. arvensis
V. calcarata
V. cenisia
V. praemorsa
V. purpurea
V. vallicola
V. becklwithii
V. sheltonii
V. rotundifolia
V. canadensis MC
V. flagelliformis
V. macloskeyi
*V. domigensis
*V. jalapaensis
V. blanda MN
V. nagasawai
V. pinnata
V. barroetana
V. pubescens
V. colvilleana
V. micrantha
V. scandens
V. capillaris
Noisettia
Hybanthus


c
outgroup
*V. kawaensis
*V. hosakai
*V. robusta
V. mayensis
V. walalealensis
V. trachelifolia
V. chamissoniana
*V. helenae
V. langsdorffii
V. affinis
V. cuculiata
V. pedata
*V. nannen
V. "nubicola"
V. gualemalensis
V. beamanii
V. hookeriana
V. humilis
*V. oxyodontis
*V. hemsleyana
V. grahamii
V. odorata GV
V. elatior
V. striata
V. reichenbachiana
*V. "percrenulata
V. adunca
V. umbraticola
V. arvensis
V. calcarata
V. cenisia
V. praemorsa
V. purpurea
V. vallicola
V. becklwithii
V. sheltonii
V. rotundifolia
V. canadensis MC
V. flagelliformis
V. macloskeyi
*V. domigensis
*V. jalapaensis
V. blanda MN
V. nagasawai
V. pinnata
V. barroetana
V. pubescens
V. colvilleana
V. micrantha
V. scandens
V. capillaris
Noisettia
Hybanthus


b
outgroup
*V. kawaensis
*V. hosakai
*V. robusta
V. mayensis
V. walalealensis
V. trachelifolia
V. chamissoniana
*V. helenae
V. langsdorffii
V. affinis
V. cuculiata
V. pedata
*V. nannen
V. "nubicola"
V. gualemalensis
V. beamanii
V. hookeriana
V. humilis
*V. oxyodontis
*V. hemsleyana
V. grahamii
V. odorata GV
V. elatior
V. striata
V. reichenbachiana
*V. "percrenulata
V. adunca
V. umbraticola
V. arvensis
V. calcarata
V. cenisia
V. praemorsa
V. purpurea
V. vallicola
V. becklwithii
V. sheltonii
V. rotundifolia
V. canadensis MC
V. flagelliformis
V. macloskeyi
*V. domigensis
*V. jalapaensis
V. blanda MN
V. nagasawai
V. pinnata
V. barroetana
V. pubescens
V. colvilleana
V. micrantha
V. scandens
V. capillaris
Noisettia
Hybanthus


d
outgroup
*V. kawaensis
*V. hosakai
*V. robusta
V. mayensis
V. walalealensis
V. trachelifolia
V. chamissoniana
*V. helenae
V. langsdorffii
V. affinis
V. cuculiata
V. pedata
*V. nannen
V. "nubicola"
V. gualemalensis
V. beamanii
V. hookeriana
V. humilis
*V. oxyodontis
*V. hemsleyana
V. grahamii
V. odorata GV
V. elatior
V. striata
V. reichenbachiana
*V. "percrenulata
V. adunca
V. umbraticola
V. arvensis
V. calcarata
V. cenisia
V. praemorsa
V. purpurea
V. vallicola
V. becklwithii
V. sheltonii
V. rotundifolia
V. canadensis MC
V. flagelliformis
V. macloskeyi
*V. domigensis
*V. jalapaensis
V. blanda MN
V. nagasawai
V. pinnata
V. barroetana
V. pubescens
V. colvilleana
V. micrantha
V. scandens
V. capillaris
Noisettia
Hybanthus

The case of Violets. Heteromorphism would have appeared recurrently in the different groups of Violets (i.e., all sections apart from section *Melanium*) as a direct consequence of changes in the ploidy level of the sporophyte. This is suggested by the small proportion of pollen heteromorphic species, 0 to 20% according to the section, and by the comparative analysis, which shows that heteromorphism is found mainly in polyploid species. Also, observations on pollen from two heteromorphic species belonging to section *Viola* (*V. reichenbachiana* and *V. hirta*) show no significant difference in germination speed and life-expectancy between 3- and 4-aperturate morphs (I. Dajoz, pers. obs.). Heteromorphic species are found in different parts of the phylogeny, often with non-heteromorphic species at their base, reflecting a derived condition. A hypothesis could be that heteromorphism disappears when polyploid species behave as diploids again, either because no selective pressure acts for its maintenance or because plants are constrained to produce one type of pollen. Such seems to be the case in the section *Plagiostigma* 2. It has a base chromosome number of x = 12 that suggests a polyploid origin, compared to the x = 6 base number of the closest section, but most species described as diploids are non-heteromorphic in that section.

The case of Pansies. No phylogeny is yet available for the section *Melanium* (Pansies). However, about the same proportion of heteromorphic species was found among diploids (75%) and polyploids (71%), which indicates no obvious link between sporophytic ploidy level and the occurrence of pollen heteromorphism in this group, as previously suspected (Dajoz et al. 1995). In the literature, ploidy levels are inferred from observations of chromosomes during meiosis. However, polyploid species may sooner or later behave as diploids (with chromosomes forming bivalents again during meiosis). Typically, this diploidization process is a continuous one, with numerous intermediary chromosomal arrangements possible between a typical tetraploid meiosis and a typical diploid one (Stebbins 1950). In *Viola*, one possible explanation for the occurrence of polyploid-non heteromorphic species and diploid-heteromorphic ones may be that, depending on the species, diploidization processes are more or less under progress. Therefore, in section *Melanium*, a possible explanation for the lack of a link between sporophytic ploidy level and pollen heteromorphism may be that all pollen-heteromorphic species of Pansies are derived from a single recent polyploid ancestor (some of them later behaving as diploids), and homomorphic species from one or several diploid ancestors. Because we lack a phylogeny of the section *Melanium*, this hypothesis cannot be ruled out, but both chromosome number and base number are highly variable among homomorphic and heteromorphic species of Pansies (Ballard 1996) and do not give obvious evidence for a diploid and a tetraploid lineage that evolved in parallel. Another possibility is that some of the species considered to be diploids are in fact recent hybrids. Indeed, frequent hybridization events are suspected to account for the high variation of chromosome number in *Melanium* (Küpfer 1974, Ballard 1996). Hybridization is normally accompanied with polyploidization and could therefore lead to variation in the aperture number of the sporophyte.

An alternative hypothesis would be that heteromorphism is maintained by natural selection in that section. A game theory model states that pollen heteromorphism is an ESS if: (i) there is a trade-off between germination speed and survival in the different pollen morphs, (ii) pollination conditions are unpre-

Fig. 3. Maximum Likelihood tree of the genus *Viola* (Ballard 1996) on which the character "pollen heteromorphism" has been mapped according to different weighting schemes. **a, b**: unweighted transition; **c, d**: transition weighted 2:1. In trees a and c, the presence of heteromorphism was considered as a derived state, whereas in trees b and d it was considered to be the ancestral state

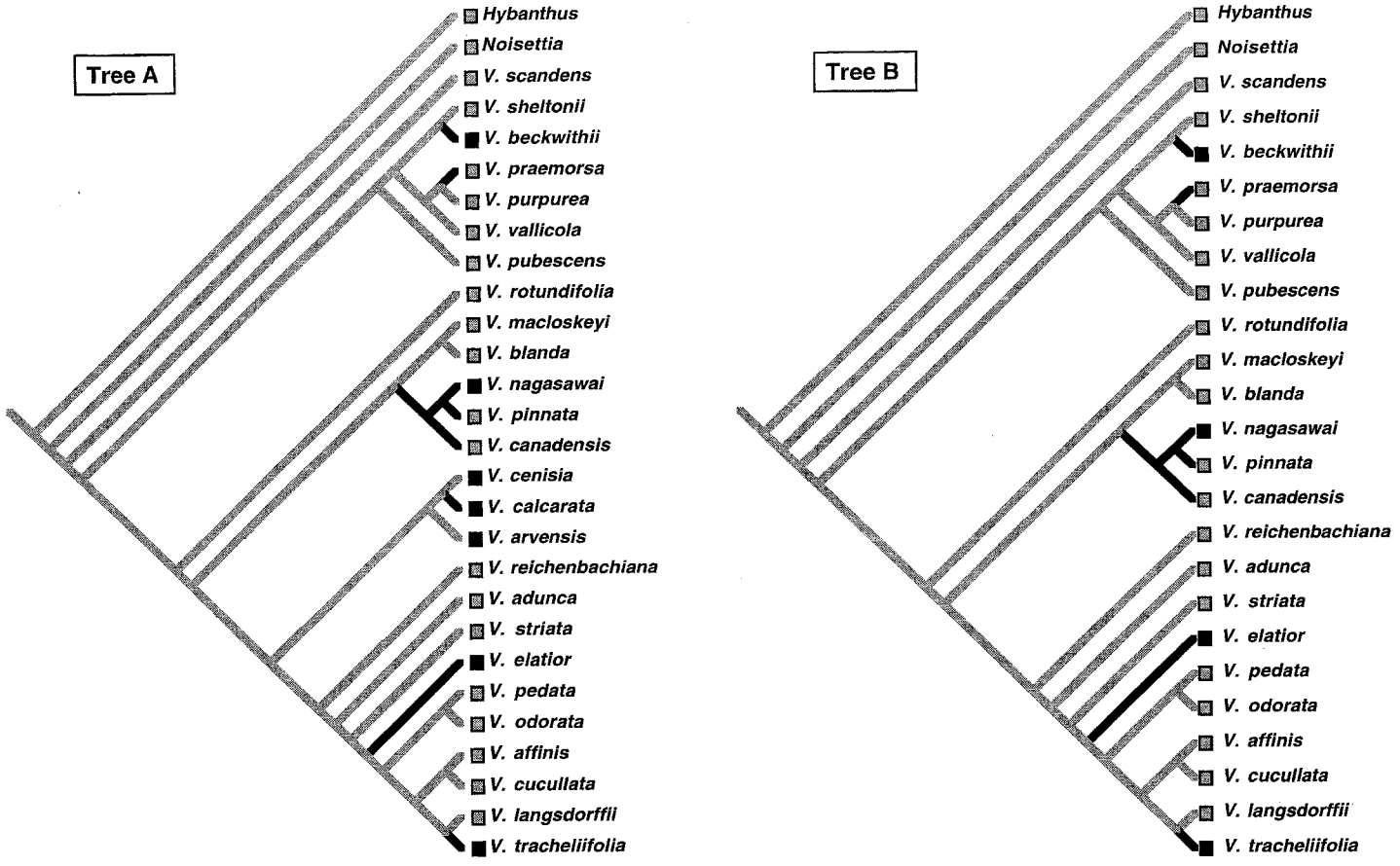


Fig. 4. Simplified phylogenies including only taxa for which data are available for both pollen and ploidy level, constructed with MacClade (Maddison and Maddison 1992). Trees A and B differ by the inclusion (Tree A) or exclusion (Tree B) of section *Melanium*.The two characters studied have been mapped on the tree. Ploidy level (independent character) has two states: diploidy (grey branches) and polyploidy (black branches). The boxes at the tip of each branch indicate the state of the dependent character: no heteromorphism (grey) or presence of heteromorphism (black)

Table 3. Contingency table established from the phylogenies in Fig. 3, including the number of each of the two states (grey and black branches) of the ploidy level (independent character), for each of the two transitions that can occur in the dependent character (pollen heteromorphism), the two states being zero (absence) or one (presence). This contingency test was done using the program CoSta (Lindefors 1997)

		Heteromorphism					
	Ploidy	$0 \rightarrow 0$	$0 \rightarrow 1$	$1 \rightarrow 0$	$1 \rightarrow 1$	Sum of branches	Fisher's exact test
Tree A	Grey	41	1	0	3	45	$P = 0.002$
	Black	5	4	0	1	10	
Tree B	Grey	40	1	0	0	41	$P = 0.0006$
	Black	5	4	0	0	9	

dictable and (iii) there is gametophytic competition on the stigma between pollen from different donors (Till-Bottraud et al. 1994). Some of these requirements are fulfilled in several species of Pansies. (i) This trade-off has been observed for 4 species of Pansies (*V. diversifolia* (Dajoz et al. 1993); *V. tricolor* and *V. arvensis* (I. Skogsmyr, unpubl. results); *V. calcarata* (Till-Bottraud et al. 1999); *V. lutea* (I. Dajoz, pers. obs.)). (ii) It is probable that pollination conditions are different between the section *Melanium* and the rest of the genus. Pansies are usually found at medium to high elevations, whereas European Violets are found in lowland habitats. This difference in the range of elevation is likely to influence pollination conditions, as it has been shown that pollination reliability decreases with elevation (Berry and Calvo 1989, Galen 1989). For example in *Viola calcarata* (a pollen-heteromorphic Pansy), the rate at which pollen was removed from the anthers significantly decreased against elevation, together with mean aperture number, suggesting that pollinator activity was less reliable at high altitudes (Till-Bottraud et al. 1999). Most Violet species bloom in early spring when pollination conditions are erratic (Beattie 1969, 1971), but they produce cleistogamous flowers that self, which limits the unpredictability of pollination. (iii) Concerning the occurrence and intensity of gametophytic competition between Violets and Pansies, observations still have to be made. However, we already know that there is a major difference in flower morphology between Violets and Pansies, involving the orientation of lateral petals. Therefore, a next step in the understanding of pollen heteromorphism is to determine whether the orientation of the lateral petals influences pollinator behaviour and frequency of visitation. If this is the case, then variation in floral traits among sections of the genus *Viola* may play a role in the evolution of pollen heteromorphism.

Conclusion

Our study has focused on the evolution of pollen heteromorphism, taking into account ploidy level variation in the sporophyte and traits of pollen ecology to account for aperture number variation. Results show that in the genus *Viola* (apart from the section *Melanium*) pollen heteromorphism is most frequent in polyploid lineages. The fate of heteromorphism depends probably upon differences in potential selective pressures such as pollination ecology. We suggest that it may be adaptive in section *Melanium*, but not in the other sections of the genus. In the future, a detailed phylogeny of section *Melanium* should shed light on the relationships between pollen-heteromorphic and homomorphic species, and enable us to determine if the few pollen-homomorphic species found in this section represent the ancestral condition or reversals.

Appendix

Species included in this study, sorted by systematic section (Ballard 1996). For each species we have indicated: the number of populations analysed (*Pop*), the mean aperture number per species (*Mean*), the variance of this mean aperture number (S^2_{ap}), the absence/presence of heteromorphism (*Het*), the base chromosome number (x), the ploidy level (*ploidy*), and the origin of the pollen samples (*data*). Species for which data have been obtained from fresh material are marked with an asterisk, the remaining data come from herbarium specimens (a: Muséum National d'Histoire Naturelle, Paris, France; b: Royal Botanic Gardens, Kew, UK; c: Natural History Museum, London, UK; d: Royal Botanic Garden, Edinburgh, UK)

Section	Species name	Pop	Mean	S^2_{ap}	Het	x	Ploidy	data
Rubellium	*V. capillaris* Gingins	5	3.0408	0.0111	no	?	?	a
Leptidium	*V. arguta* Kunth	4	3	0	no	?	?	a
	V. scandens Kunth	4	3.0137	0.0034	no	27	2	b
	V. stipularis Kunth	3	3	0	no	27	2	a
Chilenium	*V. maculata* Cav.	1	3	0	no	?	?	a
Andinium	*V. cotyledon* Gingins	2	3	0	no	?	?	b
	V. micranthella Wedd.	1	3	0	no	?	?	a
Chamaemelanium 1	*V. beckwithii* Torr. & A. Gray	1	3.235	0.1798	yes	6	4	b
	*V. biflora** L.	2	3	0	no	6	2	
	V. brevistipulata (Fr. & Sav.) W. Becker	2	3.0208	0.0102	no	6	2	a
	V. glabella Nutt.	2	3.0342	0.0165	no	6	4	a
	V. lobata Benth.	1	3.0196	0.0192	no	6	2	a
	V. nuttallii Pursh. (Maxim.)	3	3	0	no	6	4	a
	V. orientalis W. Becker	1	3	0	no	6	2	a
	V. praemorsa Douglas	1	3	0	no	6	6	b
	V. pubescens Aiton	1	3.01	0.0099	no	6	2	c
	V. purpurea Kellogg	3	3.01	0.0033	no	6	2	b
	V. sheltonii Torr.	1	3	0	no	6	2	b
	V. uniflora L.	3	3.2597	0.0706	yes	6	4	a
	V. vallicola A. Nelson	1	3.0149	0.0147	no	6	2	b
	V. xanthopethala Nakai	1	3	0	no	6	4	a
Chamaemelanium 2	*V. rotundifolia* Michx.	1	3	0	no	6	2	c
Chamaemelanium 3	*V. canadensis* L.	3	3.06	0.0219	no	6	4	b
	V. flagelliformis Heml.	2	3.0192	0.0094	no	?	?	a,b
	V. ocellata Torr. & A. Gray	1	3.0098	0.0049	no	6	2	a
Plagiostigma 1	*V. abyssinica* Steud.	3	3	0	no	?	2	a
	V. arcuata Blume	5	3.0444	0.0071	no	12	2	a
	V. betonicifolia Sm.	3	3.0526	0.0166	no	12	6	a
	V. boissieuana Makino	1	3.029	0.0281	no	12	2	a
	V. chaerophylloides (Regel) W. Becker	1	3.1765	0.1453	yes	12	2	a
	V. chinensis G. Don	1	3.041	0.0393	no	12	4	a
	V. cinerea Boiss.	1	3	0	no	?	?	a
	V. confusa Champ.	1	3	0	no	12	2	a
	V. diffusa Ging.	5	3	0	no	13	2	a
	V. distans Wall.	3	3	0	no	22	2	a

Appendix (continued)

Section	Species name	Pop	Mean	S^2_{ap}	Het	x	Ploidy	data
	V. eminii (Engl.) R.E. Fr.	4	3.1	0.0225	no	36	2	a
	V. gmeliniana Schult.	2	3.0189	0.0093	no	12	2	a
	V. inconspicua Blume	1	3.05	0.0475	yes	12	4	a
	V. jooi Janka	1	3	0	no	12	2	b
	V. mandschurica W. Becker	4	3.1456	0.0695	yes	12	4	a
	V. nagasawai Makino & Hayata	1	3.12	0.0674	yes	12	4	a
	V. oblongo-sagittata Nakai	1	3	0	no	12	6	a
	V. patrinii DC.	6	3.02	0.0025	no	12	2	a
	V. phalacrocarpa Maxim.	3	3.198	0.1061	yes	12	2	a
	V. philippica Cav.	1	3	0	no	12	2	a
	V. pinnata L.	4	3.0175	0.007	no	12	4	a
	V. selkirkii Pursh	3	3.0217	0.0071	no	12	2	a
	V. sumatrana Miq.	2	3	0	no	23	2	a
	V. takedana Makino	1	3.3478	0.2268	yes	12	2	a
	V. variegata Fisch.	6	3.03	0.0098	no	12	2	a
	V. verecunda A. Gray	2	3	0	no	12	2	a
	V. yezoensis Maxim.	3	3	0	no	12	2	a
Plagiostigma 2	*V. bissettii* Maxim.	2	3	0	no	12	2	a
	V. blanda Willd.	2	3	0	no	22	2	a
	V. epipsila Ledeb.	1	3	0	no	12	2	a
	V. lanceolata L.	1	3	0	no	12	2	a
	V. macloskeyi Lloyd	1	3	0	no	12	2	d
	V. macroceras Bunge	2	3	0	no	?	?	a
	V. vaginata Maxim.	2	3	0	no	12	2	a
Chamaemelanium 4	*V. barroetana* Schaffner	2	3	0	no	?	?	b
Xylinosium	*V. arborescens* L.	4	3	0	no	26	2	a,b
	V. scorpiuroides Coss.	1	3.2747	0.1993	yes	?	?	a
Delphiniopsis	*V. cazorlensis** Gand.	5	3.1	0.019	no	10	2	
	V. delphinantha Boiss.	2	3.0465	0.0222	no	10	2	a,b
Melanium	*V. aetolica* Boiss. & Heldr.	4	4.0294	0.0765	yes	8	2	a
	V. allchariensis G. Becker	1	3.8119	0.1527	yes	10	2	a
	V. alpina Jacq.	4	4.06	0.1064	yes	11	2	a
	V. altaica Ker-Gawl.	5	4.1461	0.1309	yes	13	4	a,b
	*V. arvensis** Murray	5	4.9	0.1901	yes	17	2	
	V. beckiana Fiala	2	3.9067	0.1006	yes	10	2	a
	V. bubanii Timb.-Lagr.	5	4.1722	0.0923	yes	13	4	a
	*V. calcarata** L.	5	4.6807	0.1703	yes	10	4	
	*V. cenisia** L.	2	3.9275	0.1858	yes	10	2	
	*V. cheiranthifolia** Humb. & Bonpl.	4	4.43	0.156	yes	?	?	
	V. comollia Massara	2	4.12	0.0821	yes	11	2	a,b
	*V. cornuta** L.	4	4.08	0.067	yes	11	2	

Appendix (continued)

Section	Species name	Pop	Mean	S^2_{ap}	Het	x	Ploidy	data
	*V. corsica** Nyman	4	4.3488	0.1108	yes	13	4	
	V. crassiuscula Bory	7	4.1221	0.0848	yes	17	2	a
	V. dacica Borb.	2	4.0172	0.013	no	13	2	a
	V. declinata Waldst. & Kit.	7	4.2157	0.0949	yes	?	?	a
	V. demetria Prolongo	1	4.0175	0.0172	no	?	?	b
	*V. diversifolia** (DC.) W. Becker	1	3.74	0.2324	yes	17	2	
	V. dubyana Burnat	2	4.0495	0.0594	yes	10	2	a
	V. dyris Maire	1	4.03	0.0291	no	?	?	a
	V. elegantula Schott	1	4.124	0.1242	yes	10	2	a
	V. fragrans Sieber	2	4.0319	0.0822	yes	?	?	a
	V. gracilis Sibth. & Sm.	5	4.29	0.1089	yes	?	?	a
	V. grisebachiana Boiss.	2	4.1818	0.1143	yes	11	2	a
	V. heterophylla Bertol. non Poir.	2	4.24	0.1534	yes	?	?	a
	V. hispida Lam.	1	4.097	0.1507	yes	17	2	a
	V. hymettia Boiss. & Heldr.	1	4.0118	0.0116	no	8	2	a
	*V. langeana** Valentine	1	4	0.0045	no	13	4	
	*V. lutea** Huds.	5	4.1382	0.0759	yes	12	4	
	V. magellensis Porta & Rigo	2	4.0476	0.058	yes	11	2	a
	V. mercurii Orph.	1	3	0	no	5	2	b
	V. munbyana Boiss. & Reuter	4	4.0918	0.1093	yes	13	4	a
	V. nebrodensis C. Presl.	1	4.0392	0.0965	yes	10	2	a
	V. nummulariifolia Vill.	1	3	0	no	7	2	a
	V. orphanidis Boiss.	5	4.6354	0.1058	yes	11	2	a
	V. orthoceras Ledeb.	2	4.23	0.1235	yes	11	2	a
	*V. palmensis** *Webb.*	1	4.0349	0.0337	no	?	?	
	V. paradoxa Lowe	2	4.0536	0.1443	yes	17	2	a
	V. parvula Tineo	2	3	0	no	5	2	a
	V. poetica Boiss. & Spruner	1	3.0395	0.0379	no	6	2	a
	V. pseudogracilis Strobl	1	4.18	0.1496	yes	17	2	a
	*V. saxatilis** F.W. Schmidt	1	4.02	0.0098	no	13	2	
	V. saxifraga Maire	1	3	0	no	13	4	a
	V. splendida W. Becker	2	4.102	0.0577	yes	?	?	a
	V. sudetica Willd.	1	4.2326	0.2715	yes	?	?	a
	*V. tricolor** L.	6	4.8313	0.1167	yes	13	2	
	V. valderia All.	2	4.4	0.2527	yes	10	2	a
Nosphinium	*V. chamissoniana* Ging.	1	3	0	no	?	?	d
	V. maviensis Mann.	2	3.04	0.0209	no	?	?	a
	V. tracheliifolia Ging.	2	3.23	0.1744	yes	10	8	a
	V. waialenalenae (J.F. Rock) Skottsb.	1	3.12	0.1056	yes	?	?	a

Appendix (continued)

Section	Species name	Pop	Mean	S^2_{ap}	Het	x	Ploidy	data
Viola	*V. acuminata* Ledeb.	4	3.02	0.0039	no	10	2	a
	V. adunca Sm.	3	3.01	0.0033	no	10	2	b
	V. affinis Le Conte	1	3	0	no	27	2	b
	*V. alba** Besser	10	3.0196	0.0026	no	10	2	
	V. ambigua Waldst. & Kit.	2	3.5319	0.0729	yes	10	4	a,b
	V. arenaria DC.	3	3.0333	0.0188	no	10	2	a
	V. beamanii Calderon	1	3	0	no	?	?	b
	*V. canina** L.	5	3.0303	0.0154	no	10	4	a,b
	V. chelmea Boiss. & Heldr.	1	3	0	no	10	2	b
	V. collina Besser	3	3.0284	0.0092	no	10	2	a,b
	*V. cucullata** Aiton	3	3	0	no	27	2	a
	V. elatior Fries	3	3.2024	0.0848	yes	10	4	a,b
	V. grahamii Benth.	1	3	0	no	?	?	b
	V. grypoceras A. Gray	3	3.037	0.0225	no	10	2	a
	V. guatemalensis Becker	1	3	0	no	?	?	b
	*V. hirta** L.	1	3.0408	0.0392	no	10	2	b
	V. hondoensis W. Becker	1	3	0	no	10	2	a
	V. hookeriana Kunth	2	3.1064	0.0475	yes	?	?	a,b
	V. humilis Kunth	1	3.0714	0.0663	yes	?	?	a
	V. jaubertiana Mares & Vigin	1	3.0156	0.0154	no	10	2	b
	V. kusanoana Makino	2	3	0	no	10	2	b
	V. langsdorffii (Regel) Fisch.	3	3.0426	0.0205	no	36	2	a
	V. mirabilis L.	1	3.0163	0.008	no	10	2	a
	V. sp. nov. ("nubicola")	1	3	0	no	?	?	b
	*V. odorata** L.	3	3.0185	0.0036	no	10	2	
	V. ovato-oblonga Maki	2	3	0	no	10	2	a
	V. pedata L.	2	3.0192	0.0063	no	27	2	a
	V. persicifolia Schreb.	7	3.0976	0.0204	no	10	2	a
	V. pumila Chaix	1	3.2763	0.2	yes	10	4	b
	*V. pyrenaica** Ramond	2	3	0	no	10	2	
	V. reichenbachiana Jordan	1	3.0198	0.0194	no	10	2	b
	*V. riviniana** Reichb	3	3.527	0.2606	yes	10	4	
	*V. rupestris** F.W. Schmidt	4	3.0471	0.0106	no	10	2	
	V. sachalinensis Boiss.	1	3.0476	0.0454	no	10	2	a
	V. sagittata Aiton	2	3.0154	0.0076	no	27	2	a
	V. senamiensis Nakai	1	3	0	no	?	?	a
	V. sieheana Becker	3	3.7755	0.0834	yes	10	4	a
	V. striata Aiton	2	3	0	no	10	2	c
	*V. suavis** M. Bieb.	5	3.05	0.0125	no	10	4	
	V. sylvestris Lam.	1	3.4107	0.242	yes	10	.	a
	V. uliginosa Besser	2	3.03	0.0246	no	10	2	a
	V. umbraticola Kunth	1	3	0	no	?	?	b
	V. wilkommii Roemer	1	3.0824	0.05	yes	10	4	a

References

Ballard H. E. (1996) Phylogenetic relationships and infrageneric groups in *Viola* (Violaceae) based on morphology, chromosome numbers, natural hybridization and Internal Transcribed Spacer (ITS) sequences. PhD Thesis, University of Wisconsin, Madison.

Ballard H. E., Systma K. J., Kowal R. R. (1999) Shrinking the violets: phylogenetic relationships of infrageneric groups in *Viola* (Violaceae) based on Internal Transcribed Spacer DNA sequences. Syst. Bot. 23: 439–458.

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

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

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

Berry P. E., Calvo R. N. (1989) Wind pollination, self-incompatibility, and altitudinal shifts in pollination systems in the high Andean genus *Espeletia* (Asteraceae). Amer. J. Bot. 76: 1602–1614.

Bronckers F. (1963) Variations polliniques dans une série d'autopolyplo artificiels d'*Arabidopsis thaliana* (L.) Heynh. Pollen et Spores 5: 233–238.

Clausen J. (1927) Chromosome number and the relationship of species in the genus *Viola*. Ann. Bot. 61: 677–714.

Clausen J. (1929) Chromosome number and the relationship of some North American species of *Viola*. Ann. Bot. 63: 741–764.

Crane P. R., Friis E. M., Pedersen K. R. (1995) The origin and early diversification of angiosperms. Nature 374: 27–33.

Dajoz I. (1999) The distribution of pollen heteromorphism in *Viola*: possible role of ploidy variations and pollination ecology. Evol. Ecol. Res. 1: 97–109.

Dajoz I., Mignot A., Hoss C., Till-Bottraud I. (1995) Pollen aperture heteromorphism is not due to unreduced gametophytes. Amer. J. Bot. 82: 104–111.

Dajoz I., Till-Bottraud I., Gouyon P. H. (1993) Pollen aperture polymorphism and gametophyte performance in *Viola diversifolia*. Evolution 47: 1080–1093.

De Fraipont M., Clobert J., Barbault R. (1996) The evolution of oviparity with egggarding and viviparity in lizards and snakes: a phylogenetic analysis. Evolution 50: 391–400.

Doyle J. A., Hotton C. L. (1991) Diversification of eraly angiosperms pollen in a cladistic context. In: Blackmore S., Barnes S. H. (eds.) Pollen and spores, patterns of diversification (Special Volume 44). Systematics Association, Clarendon Press, London.

Erdtman G. (1966) Pollen morphology and plant taxonomy. Hafner, New York, N.Y. Galen C. (1989) Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. Evolution 43: 882–890.

Goldblatt P. A. (1988) Index to plant chromosome numbers 1984–1985. Monographs in Systematic Botany from the Missouri Botanical Garden 23: 215–216.

Goldblatt P. A., Davidse G. (1981) Index to plant chromosome numbers 1975–1978. Monographs in Systematic Botany from the Missouri Botanical Garden 5: 483–486.

Goldblatt P. A., Davidse G. (1984) Index to plant chromosome numbers 1979–1981. Monographs in Systematic Botany from the Missouri Botanical Garden 8: 363–364.

Goldblatt P. A., Davides G. (1985) Index to plant chromosome numbers 1982–1983. Monographs in Systematic Botany from the Missouri Botanical Garden 13: 194–195.

Goldblatt P. A., Johnson D. E. (1990) Index to plant chromosome numbers 1986–1987. Monographs in Systematic Botany from the Mossouri Botanical Garden 30: 189–190.

Grime J. P., Hodgson J. G., Hunt R. (1986) Comparative plant ecology, a functional approach to common British species. Unwin Hyman, London.

Herrera C. M. (1990) The adaptedness of floral phenotype in a relict endemic, hawkmoth-pollinated Violet. 1. Reproductive correlates of floral variation. Biol. J. Linn. Soc. 40: 263–274.

Herrera C. M. (1993) Selection on floral morphology and environmental determinats of fecundity in a hawkmoth-pollinated Violet. Ecol. Monogr. 63: 251–275.

Herrera C. M. (1995) Microclimate and individual variation in pollinators: flowering plants are more than their flowers. Ecology 76: 1516–1524.

Hoekstra F. A. (1983) Physiological evolution in angiosperm pollen: possible role of pollen vigour. In: Mulcahy D. L., Ottaviano E. (eds.) Pollen: biology and implications for plant breed-

ing. Elsevier Science, New York, N.Y., pp. 29–33.

Knuth P. (1908) Handbook of flower pollination, vol. II Clarendon Press, London.

Kohn J. R., Graham S. W., Morton B., Doyle J. J., Barrett S. C. H. (1996) Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. Evolution 50: 1454–1469.

Küpfer P. (1974) Recherches sur les liens de parenté entre la flore orophile des Alpes et celle des Pyrénées. Boissiera 23: 1–322.

Lindefors P. (1974) Costa version 1.02 Beta. Stockholm University.

Maddison W. P., Maddison D. R. (1992) MacClade: analysis of phylogeny and character evolution. Version 3.0. Sinauer, Sunderland, Mass.

Mignot A., Hoss C., Dajoz I., Leuret C., Henry J. P., Dreuillaux J. M., Heberle-Bors E., Till-Bottraud I. (1994) Pollen aperture polymorphism in the angiosperms: importance, possible causes and consequences. Acta Bot. Gallica 141: 109–122.

Mulcahy D. L. (1979) The rise of the angiosperms: a genecogical factor. Science 206: 20–23.

Pagel M. (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. Roy. Soc. Lond. Ser. B. Biol. Sci. 255: 37–45.

Pagel M. (1997) Inferring evolutionay processes from phylogenies. Zool. Scr. 26: 331–348.

Queller D. C. (1983) Sexual selection in a hermaphroditic plant. Nature 305: 706–707.

Ressayre A., Godelle B., Mignot A., Gouyon P. H. (1998) A morphogenetic model for pollen aperture pattern in flowering plants. J. Theor. Biol. 193: 321–334.

Rolland C., Danchin E., De Fraipont M. (1998) The evolution of coloniality in birds in relation to food, habitat, predation and life-history traits: a comparative analysis. Am. Nat. 151: 514–529.

SAS (1989) SAS users's guide. Version 6 edition SAS Institute, Cary, North Carolina.

Schoen D. J., Johnston M. O., L'Heureux A. M., Marsolais J. V. (1997) Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). Evolution 51: 1090–1099.

Sillén-Tullberg B. (1993) The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. Evolution 47: 1182–1191.

Stainier F., Huard D., Bronckers F. (1967) Technique de coloration spécifique de l'exine des microspores jeunes encore groupées en tétrades. Pollen et spores 9: 367–370.

Stebbins G. L. (1950) Variation and evolution in plants. Columbia University Press, New York, N.Y.

Till-Bottraud I., Venable L., Dajoz I., Gouyon P. H. (1994) Selection on pollen morphology: a game theory model. Am. Nat. 144: 395–411.

Till-Bottraud I., Mignot A., De Paepe R., Dajoz I. (1995) Pollen heteromorphism in *Nicotiana tabacum* (Solanaceae). Amer. J. Bot. 82: 1040–1048.

Till-Bottraud I. et al. (1999) Pollen aperture heteromorphism. Variation in pollen-type proportions along altitudinal transects in *Viola calcarata*. C. R. Acad. Sci. Paris, Sciences de la vie 322: 579–589.

Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M. Webb D. A. (1968) Flora Europaea Vol. 2 (Rosaceae to Umbelliferae). Cambridge University Press, Cambridge.

Walker J. W., Doyle J. A. (1975) The base of angiosperm phylogeny: palynology. Ann. Missouri Bot. Gard. 62: 664–725.

Willson M. F. (1979) Sexual selection in plants. Am. Nat. 113: 777–790.