Evolutionary Ecology Research, 1999, **1**: 97–109

The distribution of pollen heteromorphism in *Viola*: Ecological and morphological correlates

Isabelle Dajoz*

Laboratoire d'Ecologie, CNRS UMR 7625, Bâtiment A, 7ème étage, case 237, 7 Quai St. Bernard, Université Pierre et Marie Curie, F-75252 Paris Cedex 05, France

ABSTRACT

Pollen grains have apertures that affect their life histories. Pollen heteromorphism, defined as the production by flowers of several pollen morphs that differ by their aperture number, is frequent in modern angiosperms. In species of *Viola*, it has been demonstrated experimentally that more apertures lead to faster germination, but to shorter duration of viability. The number of apertures is under genetic control and many species of *Viola* are heteromorphic for aperture number. In this study, the occurrence of pollen heteromorphism was measured in 28 species of *Viola* that belong to the European flora. The genus *Viola* is divided into two groups, violets and pansies, which differ in corolla morphology. Pollen heteromorphism was detected in more than 85% of the pansies, but in only 40% of the violets. In contrast to violets, pollen heteromorphism in pansies was not due to variation in the ploidy level of the sporophyte. In pansies, mean aperture number decreased with the elevation at which the plants were collected. This suggests that pollinator-mediated selection due to variation in pollinator fauna along the altitudinal gradient, in conjunction with other physical characteristics of the environment (temperature, potential for pollen dehydration), may affect the proportions of the different pollen types. In violets, mean aperture number increased with elevation, due to polyploid species, which exhibit pollen heteromorphism, being more abundant at higher elevation than diploid species, which are not pollen-heteromorphic. Finally, significant differences in corolla size and spur length were observed among species of pansies and, to a lesser extent, among species of violets. These differences may in part be due to differences in the pollinating fauna among the different species.

Keywords: corolla size, heteromorphism, ploidy level, pollen, pollination biology, *Viola*.

INTRODUCTION

Angiosperm plants demonstrate high variability in pollen morphology. This variation not only occurs among species (Erdtmann, 1966) but also among plants within species, reflecting both genetic and environmental effects. Intraspecific variation is found in heterostylous species where different flower types have different pollen grain morphology (Baker, 1966). In cryptically dioecious species, females have 'anthers' that produce non-viable pollen grains, whereas males have bigger viable grains (Mayer and Charlesworth, 1991). In

* e-mail: Isabelle.Dajoz@snv.jussieu.fr

nutrient-stressed plants, pollen grains can be smaller than those from control plants (Lau and Stephenson, 1993).

Within a plant, pollen morphology can vary among flowers. Such is the case in some species where cleistogamous and chasmogamous flowers co-exist on the same individual; for example, in *Collomia grandiflora*, the two flower types have different pollen morphs (Lord and Eckard, 1986). Differences in pollen grain size are also found in some chasmogamous species, within flowers (Stanton and Preston, 1986) and sometimes between early- and late-matured flowers (Thomson *et al.*, 1989).

Other than these examples, when variation of pollen grain morphology is found within a flower, such variation usually involves pollen aperture number, the aperture being the only site where the pollen tube can be initiated. Pollen grains with different aperture numbers can occur in the same flower, in all flowers of a plant and in all the plants within a population. This phenomenon is very widespread among modern angiosperms (Erdtmann, 1966; Mignot *et al.*, 1994), and has been called 'pollen heteromorphism' (Till-Bottraud *et al.*, 1995), according to the definition of heteromorphism first introduced by Venable (1985) for seeds.

The evolutionary significance of pollen heteromorphism has only recently been examined. Pollen heteromorphism is not a neutral trait because it influences pollen grain performance. For example, in several species of *Viola*, it has been demonstrated experimentally that the more apertures a pollen grain has, the more quickly it germinates. However, many-apertured pollen grains have a shorter life-expectancy (Dajoz *et al.*, 1991, 1993). Such contrasting selective pressures may act to maintain the heteromorphism. A game-theory model has shown that, depending on pollination conditions, the production by all plants within a population of several types of pollen is an evolutionarily stable strategy (Till-Bottraud *et al.*, 1994). When pollen transfer is efficient due to good environmental conditions and/or frequent visits of pollinators, high numbers of apertures should be selected. When pollen transfer is poor and/or pollinator visits are unpredictable, low aperture numbers should be favoured.

Pollen aperture heteromorphism may be associated with variation in ploidy level variation in the sporophyte and/or gametophyte. Pollen grains of polyploid sporophytes have significantly higher mean aperture numbers than pollen produced by diploids of the same species (Bronckers, 1963; Mignot *et al.*, 1994). Also, unreduced male gametophytes have more apertures than reduced ones (Cartier, 1981; Asif *et al.*, 1987). However, in four species of the genus *Viola* with heteromorphic pollen, the different pollen morphs were all found to have the same ploidy level, and variations in the proportions of these pollen morphs among plants were not correlated to variations in the ploidy level of the sporophyte (Dajoz *et al.*, 1995).

The main aim of this study was to examine the distribution and possible causes of pollen heteromorphism in the genus *Viola*. This genus is especially appropriate for such a study, since we already know that (1) several species are pollen-heteromorphic (Mignot *et al.*, 1994), (2) the different pollen morphs have different fitnesses under different environmental conditions (Dajoz *et al.*, 1993), (3) the proportions of the different pollen morphs vary among species (Dajoz *et al.*, 1995) and (4) in at least four species, the production of different pollen morphs is not due to ploidy level variations of the sporophyte and gametophyte (Dajoz *et al.*, 1995). I studied pollen heteromorphism in 28 European species of *Viola*, using both fresh plants collected from natural populations and herbarium specimens obtained from plants sampled in the field. Two main questions were addressed: First, what is the

distribution of pollen heteromorphism in the genus *Viola*? Second, is pollen heteromorphism in *Viola* correlated with variation in other floral traits, with variation in ploidy level and/or with variation in the pollinating fauna and environmental conditions influencing pollination?

METHODS AND MATERIALS

The genus *Viola* includes several hundred species in the temperate zones of both hemispheres (Beattie, 1974). *Viola* species exhibit a range of growth forms, life-cycles and breeding systems. The genus includes small shrubs or herbs, annual and perennial life-cycles (Tutin *et al*., 1968), as well as selfing and outcrossing species. Species are found in a wide array of ecological conditions, from cultivated fields and lowland woodlands to subalpine grassland and alpine ecosystems (Tutin *et al*., 1968). Flower size and the expression of secondary floral traits vary widely among species. Two subgroups have been distinguished in this genus, based on flower morphology (Fig. 1). These differences are corroborated by other characters, such as pollination ecology and habitat (H. Ballard, personal communication). Some species of violets bear chasmogamous and cleistogamous flowers on the same plant, but chasmogamous flowers mature early in the season (April–May) and cleistogamous ones later (July) (Beattie, 1969; Grime *et al.*, 1986). Pansies bear only chasmogamous flowers (Knuth, 1908; Herrera, 1993a). The reproductive system is very diversified in the genus. Obligate selfing is found only in the cleistogamous flowers of violets. All chasmogamous flowers of violets and pansies are insect-pollinated, having showy corollas with nectar guides and a spur which accumulates nectar (Beattie, 1974). Depending on the species, selfing rates range from predominant to very low (I. Dajoz, personal observation).

The survey was carried out by sampling flowering plants in the field and by asking several institutions (see Acknowledgements) for herbarium specimens of plants that had been collected in the field. I restricted the survey to European species whose taxonomic description could be found in *Flora Europaea* (Tutin *et al*., 1968). In total, 16 species of pansies and 12 species of violets were examined; these species, together with the number of populations sampled per species, are listed in Table 1. Each population was represented by five plants, whenever possible, since a few species were represented by herbarium specimens where flowers were scarce. On each plant, one flower was chosen at random. For each flower, the length of the corolla (from the tip of the left superior petal to the tip of the inferior petal;

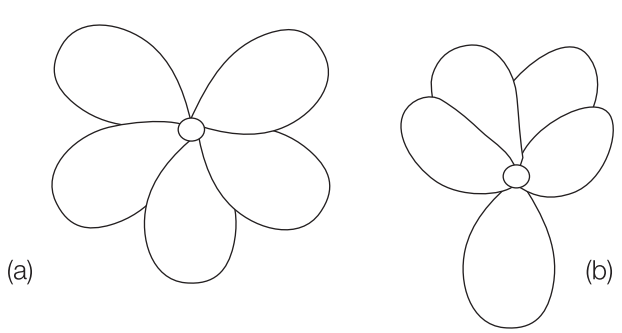

Fig. 1. Outline of corolla morphology in (a) violets and (b) pansies. Violets have corollas where the two lateral petals are directed downwards; in pansies, the two lateral petals are directed upwards.

Table 1. The species of violets and pansies investigated and the number of populations sampled per species

Pansies		Violets	
Species ($n = 16$)	Populations sampled ($n = 74$)	Species ($n = 12$)	Populations sampled ($n = 27$)
V. arborescens	1	*V. alba*	6
V. arvensis	9	*V. balearica*	1
V. biflora	1	*V. canina*	1
V. bubanii	1	*V. hirta*	2
V. calcarata	2	*V. jaubertiana*	1
V. cazorlensis	6	*V. odorata*	2
V. cheiratifolia	2	*V. palmensis*	1
V. cornuta	5	*V. pyrenaica*	1
V. corsica	1	*V. riviniana*	2
V. crassiuscula	1	*V. rupestris*	3
V. diversifolia	2	*V. stolonifera*	1
V. kitaibeliana	2	*V. suavis*	6
V. langeana	1		
V. lutea	30		
V. saxatilis	3		
V. tricolor	7		

only chasmogamous flowers being taken into account) and the length of the spur were measured. The anthers were shaken (without dissecting the flower in the case of herbarium specimens) onto a microscope slide to collect the pollen (one slide per flower). Pollen grains were stained with a drop of Alexander's stain (Alexander, 1969) and covered with a coverslip. This treatment stains the pollen wall green and the cytoplasm pink, revealing empty (and thus sterile) pollen grains. These were not common, and were discarded from our sample. On each slide, 100 filled pollen grains were counted where possible and their aperture number (range 3–6) determined. Mean aperture number of pollen grains per flower (and thus per plant, since only one flower was sampled per plant) was determined as the weighted average aperture number.

The elevation at which each plant was collected was recorded. For each species, the main pollinating agent was determined from data on the pollination biology of *Viola* in the literature (Knuth, 1908; Beattie, 1969, 1974; Herrera, 1990) and from our own field observations. The chromosome number of each species was established from the literature (Tutin *et al.*, 1968; see also Dajoz *et al.*, 1995, for a review). According to the literature, polyploid races in *Viola* are uncommon (Darlington and Janaki, 1945), and the published ploidy levels were the same within the species we investigated (Darlington and Janaki, 1945; Tutin *et al.*, 1968; Dajoz *et al.*, 1995) (see Table 1).

Statistical analysis

The mean aperture number, corolla length and spur length for each species were compared between violets and pansies with a *t*-test. For each species, in both violets and pansies, the

mean aperture number was calculated and the occurrence of one or several pollen morphs was recorded. This allowed me to detect species where pollen is monomorphic (i.e. where the main pollen morph accounts for more than 95% of all pollen grains), and species which exhibit pollen heteromorphism (i.e. where the main pollen morph represents less than 95% of all pollen grains).

In pansies, Pearson correlation coefficients were calculated to detect correlations between (1) mean aperture number per population and the elevation at which the population was sampled and (2) mean aperture number per species and chromosome number of the species to which it belongs. In violets, a Pearson correlation coefficient was computed between mean aperture number per population and the elevation at which the population had grown. Variations in mean aperture number between the two chromosomal numbers found in the species of violets I investigated ($2n = 20$ and $2n = 40$) were compared with a *t*-test.

An analysis of variance was performed to test for section (violets or pansies), species and population effects on mean aperture number per plant (with section as a fixed effect, species nested within section as a random effect and population nested within species as a random effect). An analysis of covariance was performed to test for the effect of section and pollinator type on mean aperture number per population, with elevation at which the population had been sampled and chromosome number of the species as covariates.

All statistical analyses were performed using SAS statistical software (SAS, Version 6, 1989).

RESULTS

The mean (± S.D.) pollen aperture number differed significantly between violets (3.16 ± 0.31, $n = 12$) and pansies (3.89 ± 0.52, $n = 16$) ($t_{26} = 4.26$, $P < 0.0002$).

Values of mean aperture number for each one of the 28 species investigated are given in Table 2. Here, a species is defined as heteromorphic if the most abundant pollen morph represents less than 95% of all the pollen grains counted. The data show that pollen heteromorphism is much more widespread in pansies (13 of 16 species investigated or 81.25% were heteromorphic) than in violets (5 of 12 species sampled or 41.7% were heteromorphic). This difference is significant (Fisher's exact test, $P = 0.038$).

In pansies, there was no significant correlation between mean aperture number of a species and its chromosomal number ($r = -0.16$, d.f. = 13, $P = 0.59$). On the other hand, in violets, the mean aperture number was significantly lower in species with $2n = 20$ (3.01 ± 0.03) than in species with $2n = 40$ (3.19 ± 0.27) ($t_{20} = -3.01$, $P < 0.007$). This suggests that ploidy level plays a role in the occurrence of pollen heteromorphism in violets.

In pansies, the elevation at which the plants were found influenced mean pollen aperture number: a significant negative correlation was found between elevation and mean aperture number per population ($r = -0.26$, d.f. = 67, $P = 0.03$; Fig. 2). In contrast, in violets, there was a significant positive correlation between elevation and population mean aperture number ($r = 0.44$, d.f. = 20, $P = 0.05$).

There were highly significant differences in mean aperture number among the two sections of the genus *Viola*; this was also the case among species within sections and among populations within a species (Table 3).

Most of the variation observed at the population level in mean aperture number was among sections, but chromosome number and pollinator type effects were also significant (Table 4). For example, in pansies, species pollinated by bumblebees had a higher mean

Table 2. Mean corolla length, spur length and aperture number, presence of pollen heteromorphism, mean proportions of the different pollen morphs, chromosome number and main pollinating agent for each of the 28 species of violets and pansies investigated

Species	Mean corolla length (mm)	Mean spur length (mm)	Mean aperture number	Heteromorphism (mean proportions of the different pollen morphs)	Chromosome number ($2n$)	Main pollinating agent
Violets						
V. alba	15.96	4.95	3.00	no (100% 3)	20	bees
V. hirta	N.A.	N.A.	3.01	no (98.6% 3; 1.4% 4)	20	bees
V. odorata	17.25	4.75	3.00	no (100% 3)	20	bees
V. suavis	16.54	4.29	3.01	yes (92.7% 3; 7.3% 4)	40	bees
V. canina	15.33	6.66	3.39	yes (66% 3; 34% 4)	40	bumblebees
V. riviniana	21.50	7.00	3.44	yes (60.7% 3; 39.3% 4)	40	bumblebees
V. balearica	14.00	3.50	3.00	no (100% 3)	?	?
V. jaubertiana	12.75	1.75	3.08	no (98% 3; 2% 4)	20	?
V. palmensis	19.80	6.80	3.13	yes (93.5% 3; 6.5% 4)	?	?
V. pyrenaica	14.50	3.00	3.00	no (100% 3)	20	?
V. rupestris	13.44	3.84	3.01	no (99.2% 3; 0.8% 4)	20	?
V. stolonifera	15.00	N.A.	3.00	no (100% 3)	?	?

Pansies						
V. arvensis	10.17	3.55	4.70	yes (1.8% 3; 23.9% 4; 73.3% 5; 1% 6)	24	bumblebees
V. lutea	16.05	5.31	3.97	yes (6% 3; 90.7% 4; 3.3% 5)	48	bumblebees
V. saxatilis	17.10	4.10	3.96	yes (5% 3; 90% 4; 5% 5)	26	bumblebees
V. tricolor	12.84	4.88	4.24	yes (1.6% 3; 72.2% 4; 25.9% 5; 0.3% 6)	26	bumblebees
V. biflora	11.67	1.58	3.07	no (99.2% 3; 0.8% 4)	12	flies
V. bubanii	27.50	10.50	3.88	yes (8.9% 3; 91.1% 4)	128	Lepidoptera
V. calcarata	20.22	7.64	4.05	yes (2.2% 3; 89.3% 4; 7.9% 5; 0.5% 6)	40	Lepidoptera
V. cazorlensis	13.00	20.44	3.01	yes (95% 3; 5% 4)	20	Lepidoptera
V. cornuta	16.90	22.19	3.86	yes (9.7% 3; 88.2% 4; 2% 5)	22	Lepidoptera
V. corsica	10.97	10.97	4.03	yes (2% 3; 95% 4; 3% 5)	52	Lepidoptera
V. diversifolia	18.50	5.75	3.66	yes (33% 3; 67% 4)	34	Lepidoptera
V. arborescens	8.30	2.80	3.00	no (100% 3)	140	?
V. cheiratifolia	14.30	6.04	4.19	yes (82.6% 4; 17.4% 5)	?	?
V. crassiuscula	8.17	1.67	4.02	yes (1.3% 3; 95% 4; 3.7% 5)	?	?
V. kitaibeliana	6.12	2.33	3.91	yes (64% 3; 21% 4; 15% 5)	16	?
V. langeana	14.17	5.00	3.99	no (0.5% 3; 99.5% 4)	?	?

Note: N.A. = no measurements available, ? = no information available.

Table 3. Results of an analysis of variance performed to test for section (violets or pansies), species and population effects on mean aperture number per plant, with section as a fixed effect and species nested within section (Sp(section)) and population nested within species (Pop(sp*section)) as random effects

Trait	d.f.	MS	*F*	*P*
Section	1	13.95	16.78	0.0003
Sp(section)	26	1.76	25.13	0.0001
Pop(sp*section)	74	0.11	6.28	0.0001
Error	302	0.017		

aperture number than those pollinated by Lepidoptera (4.07 *vs* 3.47); in violets, species pollinated by bumblebees had a higher mean aperture number than those pollinated by bees (3.42 *vs* 3.01). On the other hand, there was no significant effect of elevation on mean aperture number per population, but this may have been because there was a negative correlation between elevation and mean aperture number per population in pansies, whereas the correlation was positive in violets. However, the interaction between elevation and section was not significant (Table 4). This means that the differences in mean aperture number between populations that grow at different elevations are not as important as those due to differences in chromosome number or pollinator type, and that a larger sample size might be needed to detect them.

DISCUSSION

Pollen heteromorphism is not homogeneously distributed in the genus *Viola*. Pollen heteromorphism was found in more than 80% of the species of pansies examined, but

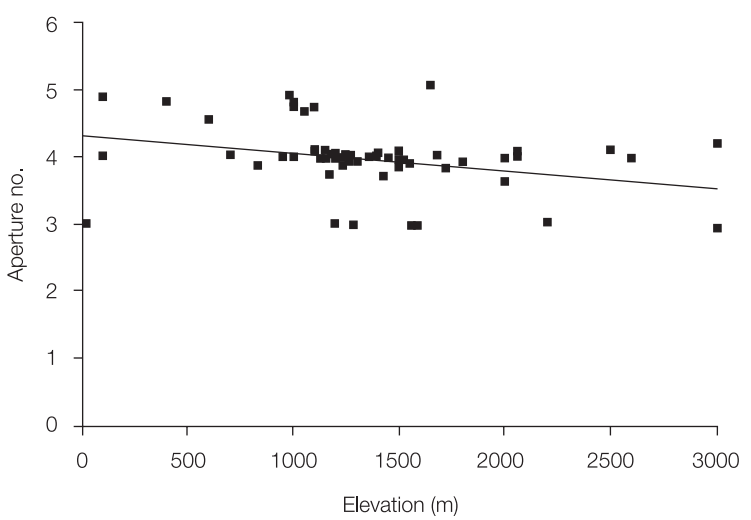

Fig. 2. Plot of mean aperture number per population versus elevation at which the population grew for pansies. There was a significant negative correlation between these two variables: $r = -0.26$, d.f. = 67, $P = 0.03$.

Table 4. Results of an analysis of covariance performed to test for the effect of section (Tax) and pollinator type (Poll) on mean aperture number per population, with elevation at which the population had been sampled (Alt) and chromosome number (Nbch) of the species as covariates

Trait	d.f.	MS	*F*	*P*
Alt	1	0.02	0.25	0.61
Nbch	1	1.32	16.42	0.0001
Tax	1	10.01	124.14	0.0001
Poll	2	1.46	18.17	0.0001
Nbch*Tax	1	0.013	0.17	0.68
Alt*Tax	1	0.088	1.09	0.29
Nbch*Poll	1	2.14	26.55	0.0001
Alt*Poll	2	0.58	7.25	0.0015
Error	64	0.08		

in only 41% of the species of violets sampled. Furthermore, mean aperture number in violets was very close to 3, because most of the species examined produced only (or mainly) 3-aperturate pollen grains. In contrast, in pansies, the mean aperture number was much higher: 3-, 4-, 5- and 6-aperturate pollen grains were found in this group, sometimes within the same species and even the same plant, since pollen heteromorphism is widespread.

Pollen heteromorphism in *Viola* was not due solely to variation in ploidy level of the sporophyte. In the species of pansies examined, there was no significant correlation between mean aperture number and chromosome number. More strikingly, of the three species of pansies that exhibited no pollen heteromorphism, one species (*V. biflora*) has the lowest chromosome number ($2n = 12$; Tutin *et al.*, 1968) of all European members of the genus, and another, *V. arborescens* ($2n = 140$; Tutin *et al.*, 1968), has the highest. This further supports the hypothesis that, in pansies, pollen heteromorphism is not due to variations in ploidy level. In contrast, in violets, there is a statistically significant correlation between mean aperture number and chromosome number. In species where $2n = 20$, mean aperture number is very close to 3. In species where $2n = 40$, mean aperture number is higher, but these species produce only 3- and 4-aperturate pollen grains. It would thus appear that, in pansies and violets, the origin of pollen heteromorphism may be different.

In the literature, several authors have reported species in which pollen heteromorphism is associated with ploidy level variation of the sporophyte (Bronckers, 1963; Erdtmann, 1966), as found in the present study for violets. An important point is that more than 80% of all angiosperm species are considered to be ancient polyploids (Stebbins, 1971). According to Küpfer (1974), pansies have a common ancestor whose chromosome number was 14, and all modern species are derived from this basic chromosome number, either by polyploidization, amphiploidization or hybridization. All species of pansies sampled in this study behave as diploids (Tutin *et al.*, 1968; Dajoz *et al.*, 1995), even though they might have originated by polyploidization. Therefore, it is possible that pollen heteromorphism in pansies has appeared after some polyploidization events, but other selective forces must have acted on this heteromorphism to maintain it and make it evolve, in order to obtain the situation that holds today.

A phylogeny of the genus *Viola* (Clausen, 1929) was based on the variation of chromosome number among species. According to this phylogeny, two branches arise from a section that contains the most primitive species of the genus: one of them (Rostellatae-Nominium section) contains only violets and the other (Melanium section) contains only pansies. Both pollen-heteromorphic and pollen-monomorphic species are found in all three subsections of Section Rostellatae-Nominium, probably because Clausen (1929) put species with $2n = 20$ and $2n = 40$ in each of these three subsections. Clausen (1929) believed section Melanium to be recent; therefore, this section would contain the most modern species of the genus *Viola*. All or almost all the species investigated in this section are pollen-heteromorphic.

On the whole, these data support the hypothesis that, in violets, the occurrence of pollen heteromorphism can be linked to variations in the ploidy level of the sporophyte, but only 3- and 4-aperturate pollen grains are produced. In pansies, it is not possible to correlate mean aperture number and chromosome number, and the range of variation of aperture number is much greater (from 3 to 6), suggesting that selective pressures could act on this trait. However, this does not mean that, in the past, pollen heteromorphism has never been linked to ploidy level variations of the sporophyte. One aim in the near future is to use a molecular phylogeny of the genus *Viola* (Ballard, 1996) to determine precisely how many times pollen heteromorphism arose in the genus.

The effect of elevation at which the plants grew had different effects on mean aperture number in violets and pansies: mean aperture number decreased with elevation in pansies, but increased with elevation in violets. All members of the genus *Viola* are insect-pollinated (Knuth, 1908; Beattie, 1974). Therefore, possible selective pressures acting on pollen heteromorphism may be due to differences in pollinator activity (Till-Bottraud *et al*., 1994), because the different pollen morphs in *Viola* have different fitness in different conditions. The variation in some environmental parameters might therefore correlate with the variation in the proportions of the different pollen morphs, if these parameters influence the behaviour of pollinators. It is widely acknowledged that physical environmental factors can influence the activity of pollinators (Herrera, 1995) and that this will in turn affect plant reproductive success (because variation in pollen deposition and export acts both on male and female components of fitness). The environmental parameter studied here was elevation. Shifts from bee and bumblebee to butterfly and fly pollinations are observed along altitudinal gradients throughout the world: in the Andes, in the Rocky mountains of North America, in New Zealand, the Himalayas and in the European Alps (Arroyo *et al.*, 1982, and references therein; Herrera, 1995). Bees and bumblebees are less abundant at high elevation, perhaps because they need to collect food from flowers not only to sustain themselves, but also to feed their larvae. Rearing larvae requires a consistent and continuous supply of both pollen and nectar. Uncertainty in weather conditions can restrict pollinator activity. Also, butterflies and flies are more efficient than bees and bumblebees at warming themselves in the sun or using the heat emitted by flowers (Arroyo *et al.*, 1982). Therefore, it is logical to observe a shift in mean pollinating agent with elevation, and the data obtained here on pansies support this hypothesis.

Pollination activity also seems to be affected by elevation. A study carried out on the genus *Espeletia* (Compositae), which occurs over a wide altitudinal range in the Andes, showed that visitation rates of pollinators strongly decrease with elevation. For example, in the species which grow at elevations over 4000 m, pollination rates were so low that plants were visited only once every 35–167 h (Berry and Calvo, 1989). A similar trend has been

observed for pollinators of wild jasmine in the Southern Pyrenees (J.D. Thompson, personal communication). Finally, Lepidoptera and flies may not be as active as bumblebees and bees, since they do not need to forage for the nest. For example, in *V. cazorlensis*, which is mainly pollinated by hawk moths and grows in middle-elevation mountains of Spain, an average of 0.6 moth visits per hour was recorded in a population of plants that comprised about 100 individuals (Herrera, 1990), but seed set was satisfactory because of the long lifespan of the flowers (Herrera, 1993a) and possibly because of the long viability of pollen grains (since *V. cazorlensi*s produces mainly 3-aperturate pollen grains; Table 1). The combined effects of a shift in pollinator taxa and decrease in visit frequency at higher elevation may select for longer viability of pollen. This may explain why mean aperture number decreases with elevation in pansies, since lower aperture number in the genus is correlated with increased longevity (Dajoz *et al*., 1993).

In violets, there is an increase in aperture number with elevation, but the mean aperture number also depends on the chromosome number of the mother plant. Species with $2n = 20$ are not heteromorphic, whereas species with $2n = 40$ often are. The increase in aperture number with elevation may thus be due to the fact that species with $2n = 20$ grow at low elevations, and species with $2n = 40$ grow at high altitudes. For species with $2n = 20$, the mean elevation where plants grew was 685 m; for species with $2n = 40$, the mean elevation where plants grew was 1191 m; this difference was statistically significant ($t_{49} = 4.13$, $P < 0.0001$). In the literature, there is evidence that ploidy level increases with elevation (Stebbins, 1971) and also with latitude (see Rosenzweig, 1995, for a review). In violets, the increase in aperture number with elevation could be the result of an increase in chromosome number with elevation. Furthermore, the altitudinal range over which violets are found is much smaller that in pansies, and this may not be enough for shifts in pollination modes to occur.

In conclusion, patterns of pollen aperture number variation in various species of *Viola* include both ecological and chromosomal factors. As predicted by the theory (Till-Bottraud *et al*., 1994), some ecological factors that influence pollination efficiency are also linked to the variations in the proportions of the different pollen morphs. Also, significant variations in corolla size and spur length are observed among species, in both violets and pansies, and the data in the literature suggest that, depending on the pollinating agent, different corolla traits will influence the fitness of a plant (Galen, 1989; Klinkhamer *et al.*, 1989; Young and Stanton, 1990; Campbell *et al*., 1991; Herrera, 1993b). Therefore, in pollen-heteromorphic species of *Viola*, we may expect to find correlations between traits of corolla morphology, pollinator visitation frequency and pollen mean aperture number. Within pollen-heteromorphic species of *Viola*, differences among populations have already been observed in flower morphology and mean aperture number (Dajoz and Till-Bottraud, 1992; Dajoz *et al*., 1993). The next step in the understanding of the evolution of pollen heteromorphism will be to identify how selective pressures due to differences in pollinator behaviour, mediated through flower morphology, can act on pollen aperture number and morphology.

ACKNOWLEDGEMENTS

Many thanks to J. Shykoff and J.D. Thompson for constructive criticism of the manuscript; M. Lachaud, whose help in counting the pollen slides was precious; and M. Lefranc, who collected specimens of *V. calcarata* in the field. The comments of M.L. Rosenzweig and two anonymous reviewers were very helpful in improving the manuscript.

Many thanks are also due to the following institutions and colleagues, who kindly collected samples in the field and/or lent out herbarium specimens: Dr A. Aboucaya, Conservatoire Botanique de Porquerolles, Parc National de Port-Cros, Hyères, France; Dr M. Amézquita, Departamento de Biologia Vegetal, Universitat de València, Spain; Dr M. Clemente, Jardin Botanico de Cordoba, Cordoba, Spain; Dr J.L. Gradaille, Jardi Botanic de Soller, Museu Balear de Ciencies Naturals, Soller, Balears, Spain; Dr C. Herrera, Estacion Biologica de Donana, Consejo Superior de Investigaciones Cientificas, Sevilla, Spain; Dr M. Jafari, Research Institute of Forests and Rangelands, Education and Research Division, Tehran, Iran; Dr R. Verlaque, Biosystématique et Ecologie Méditerranéenne, Université de Provence, Marseille, France; and Drs L. Villar and D. Gomez, Herbarium of Jaca, Instituto Pyrenaico de Ecologia, Consejo Superior de Investigaciones Cientificas, Jaca, Spain.

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