STUDIES IN THE POLLINATION ECOLOGY OF *VIOLA*.
I. THE POLLEN-CONTENT OF STIGMATIC CAVITIES

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Abstract

Samples of chasmogamous flowers of three species of *Viola* from seven different habitats were examined for the contents of their stigmatic cavities. Flower and pollen morphology are described. Insect exclusion experiments suggest that self-pollination is rare and emasculations have established that intraspecific and interspecific pollen transfer is frequent. The quantity of pollen introduced into the stigmatic cavity is affected by several factors. Rain reduces pollination in exposed habitats and similar decreases are detected in sheltered or north- or east-facing habitats due to the smaller amount of sunlight incident upon them. Percentage pollination remains high in habitats with no blossoms of other genera; anthophilous insects visit *Viola* for food. Habitats with a variety of blossoms besides *Viola* may lure sufficient insects to ensure the pollination of all species, including the inconspicuous ones. However, large stands of showy blossoms may monopolize the insect visitors in a habitat also occupied by less attractive species. It is argued that gene flow in disturbed or marginal habitats may be increased by the greater availability of potential pollinators.

Introduction

The three species chosen for the present study, *Viola riviniana* Rchb., *V. reichenbachiana* Jord., and *V. hirta* L., produce chasmogamous flowers in spring and cleistogamous flowers in summer. The chasmogamous flowers are morphologically complex and are manifestly entomophilous (see page 143). They are self-compatible (Valentine 1962), although experiments on *V. riviniana* described below suggest that self-pollination is rare, a point reported also by Sprengel for *V. odorata* L., by Darwin for *V. canina* L. (both in Knuth 1908), and for *V. mirabilis* L. (Kerner & Oliver, 1902). Further experiments on *V. riviniana*, also reported below, suggest that the pollination mechanism is highly functional, and personal observations confirm that the chasmogamous flowers of all three species are capable of producing viable seed. Several authors, notably Schmiedeknecht (1878, 1930), Müller (1883) and Knuth (1908), have reported that the flowers are visited by a variety of insects. Despite these conditions, however, they frequently fail to set seed, a fact recorded for *V. mirabilis* (Kerner & Oliver 1902), *V. odorata* and *V. riviniana* (West 1930), *V. hirta* (Knuth 1908) and subsection *Rostratae* Kupffer (Gregory 1912).

It seems reasonable to suppose that the failure to set seed in the chasmogamous flowers of *Viola* may be due mainly to the breakdown in the transference of pollen. This process depends upon the distribution and availability of suitable vectors, and these in turn are affected by a large number of ecological factors. Four such factors are considered in this paper, namely weather, amount of shade in the habitat, direction of slope of the habitat and the proximity of other blossoms of the same, or of different species.

Materials and methods

Material was gathered from seven different habitats in the chalk countryside of southern England. Six of these habitats occurred within an area of approximately 30 hectares (80 acres) of woodland and parkland near Winterslow, Wiltshire, and the seventh was an exposed hillside, known as Juniper Top, near Dorking in Surrey. A small sample of white-flowered *V. odorata* was collected from an eighth habitat in the Winterslow locality, but will not be considered with the main bulk of data.

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Fifty flowers from one colony within each habitat were picked and preserved in a 9 : 1 mixture of 70 per cent. ethanol and glycerol. This viscous fluid minimized movement of loose pollen amongst the specimens. Care was taken to choose flowers in mid-anthesis, a condition recognized by the presence of unwrinkled corollas of fresh appearance and glistening at the mouth of the corolla-tube, borne by pedicels sharply hooked at the distal end.

In the laboratory specimens were dissected, and the style removed and transferred to glycerine jelly stained to the colour of claret by the addition of basic fuchsin. This mountant left the stylar tissue greenish-yellow in colour, but stained pollen grains red.

The chief topographical and floral features of each habitat are summarized in Table 1.

FLOWER AND POLLEN MORPHOLOGY

Flowers

The accounts of visitors to the chasmogamous flowers of *Viola* referred to in the introduction reveal that hover-flies (Diptera: Syrphidae), bee-flies (Diptera: Bombyliidae) and bees (Hymenoptera: Apoidea) are most frequently attracted to them. The flowers may thus be termed myophilous (fly-loving) and melittophilous (bee-loving). Pollination syndromes of flowers of this type include characters such as zygomorphy, pale blue to violet colouring, moderately deep corolla-tubes, distinct nectar-guides, diurnal pollen and nectar presentation, nectar and pollen open or fairly effectively concealed, and a sweet odour, (Baker & Hurd 1968, Faegri & van der Pijl 1966). These characters are present to a greater or lesser degree in the three species under discussion.

Fig. 1. shows that the size and arrangement of organs differ slightly between the species, particularly the styles, which are illustrated by Fig. 2. During anthesis the ovary is completely enclosed by the ring of stamens and much of the style is similarly hidden by the cone of connective appendages. The tip of the style is the only part of the gynoecium visible in the open flower, and bears short papillae, except in *V. hirta*. The number and size of these papillae are greater in *V. reichenbachiana* than in *V. riviniana* although much variation is found in both species.

The style is tubular with a lumen which is continuous with the cavity of the ovary but for a constriction near the base. Its interior walls stain deeply with PAS (periodic-acid Schiff), and secrete mucilage which so fills the lumen that the stigma-opening can be seen to be moist. The stigmas are absent as external receptive surfaces so pollen germinates only with the lumen of the style. The style pivots upon the distinct area of flexure near its base. The tissues in this region are elastic hence the style may be strongly deflected by a visiting insect, but immediately regains its normal position once the visit has ended.

Observations with fresh flowers in the laboratory show that during early anthesis movement of the style causes no visible changes at the opening of the stigmatic cavity. At full anthesis, however, disturbance of the style causes the exudation of a drop of mucilage at the stigma-opening. In an attempt to determine the possible function of this phenomenon, a small camel-hair brush loaded with violet pollen was repeatedly drawn over the stigma-opening at the same time as the style was raised with a mounted needle. In newly-opened flowers the majority of the pollen merely fell on to the anterior petal. However, in flowers two to three days old, a high proportion of grains adhered to the stigma-opening, presumably as a result of the sticky nature of the mucilage.

McLean & Ivimey-Cook (1956) suggest that disturbance of the style closes the lumen in the area of flexure hence increasing the pressure of mucilage within. As a consequence of this a drop is exuded from the stigma-opening and any pollen in the vicinity adheres to it. Then, when the style springs back into place, the basal lumen resumes its normal shape and capacity, the mucilage drop is withdrawn into the stigmatic cavity and pollination is effected. MacLeod, in Knuth (1908), suggested that the mucilage was deposited upon visiting insects to form a moist and viscous area suitable for the reception of the dry, powdery pollen. The results of my own observations rather favour the former explanation

TABLE 1. Chief topographical and floral features of habitats. Shelter denotes the shade afforded by the dominant plants, the symbols indicate: + much shade, — shade only when sun low in arc, 0 none. Distribution of blossoms (N.B. not plants) within a 20 square-yard area indicated: (a) large clumps; (b) small clumps; (c) singly; (d) evenly.

Site No.	Height Above Sea Level (feet)	Direction of Slope	General Description and Grid Reference	Type	Soil Drainage	Cover	Dominant Plants	Shelter	Blossoms	Distribution
A	350–370	NE	Edge of wood SU 251 338	15 cm clay with flints on chalk	Good excepting after wet weather	15 cm leaf mould	*Quercus robur* *Crataegus monogyna* *Rubus fruticosus*	+ + 0	*Viola reichenbachiana* *Mercurialis perennis*	a, b, c.
B	345–355	NE	Edge of wood SU 253 337	As site A	As site A	15 cm leaf mould	As site A		*Viola riviniana* *Endymion non-scriptus* *Anemone nemorosa* *Primula vulgaris* *Taraxacum officinale*	b, c, a c b c
C	440–410	NE	Glade in wood SU 255 335	As site A	As site A	100 per cent. grass and tussocks	Graminae	0	*Viola hirta* *Viola riviniana* *Ajuga reptans* *Taraxacum officinale*	b, c c c c
D	350–370	W	Slope between wood and field SU 254 339	5–10 cm clay on chalk	Good	100 per cent. grass and tussocks, some *Mercurialis* stands	Graminae *Fagus sylvatica* at edge	0	*Viola reichenbachiana* *Viola hirta* *Glechoma hederacea* *Primula vulgaris* *Mercurialis perennis* *Fragaria vesca*	b b, c b, c a, b a c

E	390	—	In wood near a ride SU 254 339	30 cm clay on chalk	Poor	10 cm leaf mould	*Quercus robur*	+	*Viola reichenbachiana*	b
							Corylus avellana	+	*Endymion non-scriptus*	b
							Rubus fruticosa	0	*Primula vulgaris*	b
									Adoxa moschatellina	b
									Lathraea squamaria	b
F	350	S	Coppiced wood SU 254 339	As site E	Fair	Patchy leaf mould, much bare soil	*Corylus avellana*		*Viola riechenbachiana*	a, b
							cut 2 years		*Viola riviniana*	a, b
							previously.	—	*Endymion non-scriptus*	b
									Primula vulgaris	a, b
							Occasional:		*Glechoma hederacea*	b
							Quercus robur	—	*Anemone nemorosa*	b
							Betula pendula	—	*Orchis mascula*	b, c
									Fragaria vesca	b
									Bellis perennis	b
									Ranunculus ficaria	c
G	300	—	Tree stand in horse paddock SU 254 342	As site E	Fair	Bare soil	*Fagus sylvatica*	+	*Viola odorata*	a
H	440–460	W	Exposed chalk hill TQ 182 527	Thin clay on chalk	Good	Very scant leaf-mould many bare patches	Graminae	0	*Viola hirta* subsp. *hirta*	b, c
									Viola hirta subsp. *calcarea*	c
									Viola riviniana subsp. *riviniana*	b, c
									Viola riviniana subsp. *minor*	c
									Taraxacum officinale just outside habitat	c

although I believe both to be valid. The drop of mucilage seems to increase the area to which pollen grains may adhere. The opening of the stigmatic cavity is very small and any mechanism that increases the chance of pollen passing through it would increase the efficiency of the pollination mechanism.

Pollen

It is impossible to distinguish between the pollen grains of the three species of *Viola* under study on the basis on morphology alone. The following description is therefore valid for all three species, with some additions for *V. riviniana.*

The pollen is powdery, and when examined fresh the grains are ellipsoidal to spheroidal with the colpi appearing as narrow grooves. When moistened for more critical examination they frequently become entirely spheroidal and the colpi may gape widely. There are three colpi arranged equidistantly from one another around the equator, with their longitudinal axes meridionally directed. They are very slightly biased towards one pole, hence in microscopical preparations a number of differing shapes can be seen. In surface view the exine lacks any distinguishing morphological features; it is smooth and very finely granular in appearance, without a trace of a reticulum.

V. riviniana has tricolpate and tetracolpate grains. A further distinction can be made according to size and exine thickness, there being small, thick-walled and large, thin-walled grains, a feature first reported by West (1930).

A character which helped to distinguish *Viola* pollen from that of other genera encountered in this study was its tendency to take up basic fuchsin stain more slowly. In a fresh preparation they would invariably appear paler than all other grains, with the colouring concentrated in the walls but absent at the colpi. The only character that appeared to vary significantly between *Viola* species was size, a feature manifest in the diameter of the polar view. Samples were measured in an attempt to demonstrate differences sufficient to separate the species.

The first series of samples were from the habitats under study and four species were investigated: *V. riviniana*, *V. reichenbachiana*, *V. hirta* and *V. odorata.* Eight preparations were made, two per species, each from a single flower and a separate plant. The dimension measured was the diameter of the polar view. An analysis of variance on the data, followed by the calculation of the least significant difference, showed significant differences in pollen diameter between species, except *V. hirta* and *V. odorata.* However, the difference between *V. riviniana* and *V. reichenbachiana* was so small as to suggest that the pollen of these species found in the stigmatic cavities would in practice be indistinguishable. In order to settle the matter a second series of samples were measured, and this time three flowers of each species from widely separated localities in Europe were used. Significant differences in diameter now appeared only between the two pairs of species, *i.e.* between *V. riviniana* + *V. reichenbachiana* and *V. hirta* + *V. odorata.* Pollen grains at either size extreme could be named with some confidence, but generally the character was not used because of its unreliability. Actual figures for the polar view diameter were $30 \cdot 5\mu$–$44 \cdot 2\mu$ for *V. riviniana* and *V. reichenbachiana*, and $25 \cdot 5\mu$–$34 \cdot 5\mu$ for *V. hirta* and *V. odorata.*

INSECT EXCLUSION EXPERIMENTS AND EMASCULATIONS

The experiments described here were carried out at habitats B (edge of wood) and C (glade in wood) in Wiltshire in the spring of 1966 on *V. riviniana.*

Insect exclusion experiments

Small polythene bags were placed over thirty individual plants from which all open flowers had been removed. Each bag, 19 × 21 cm, had a sealing mechanism at one end and this allowed the plant within to bloom freely while excluding airborne insects.

Eight days later the flowers were picked and preserved. In the laboratory the styles were mounted and examined for pollen. Twenty five out of thirty specimens bore no traces

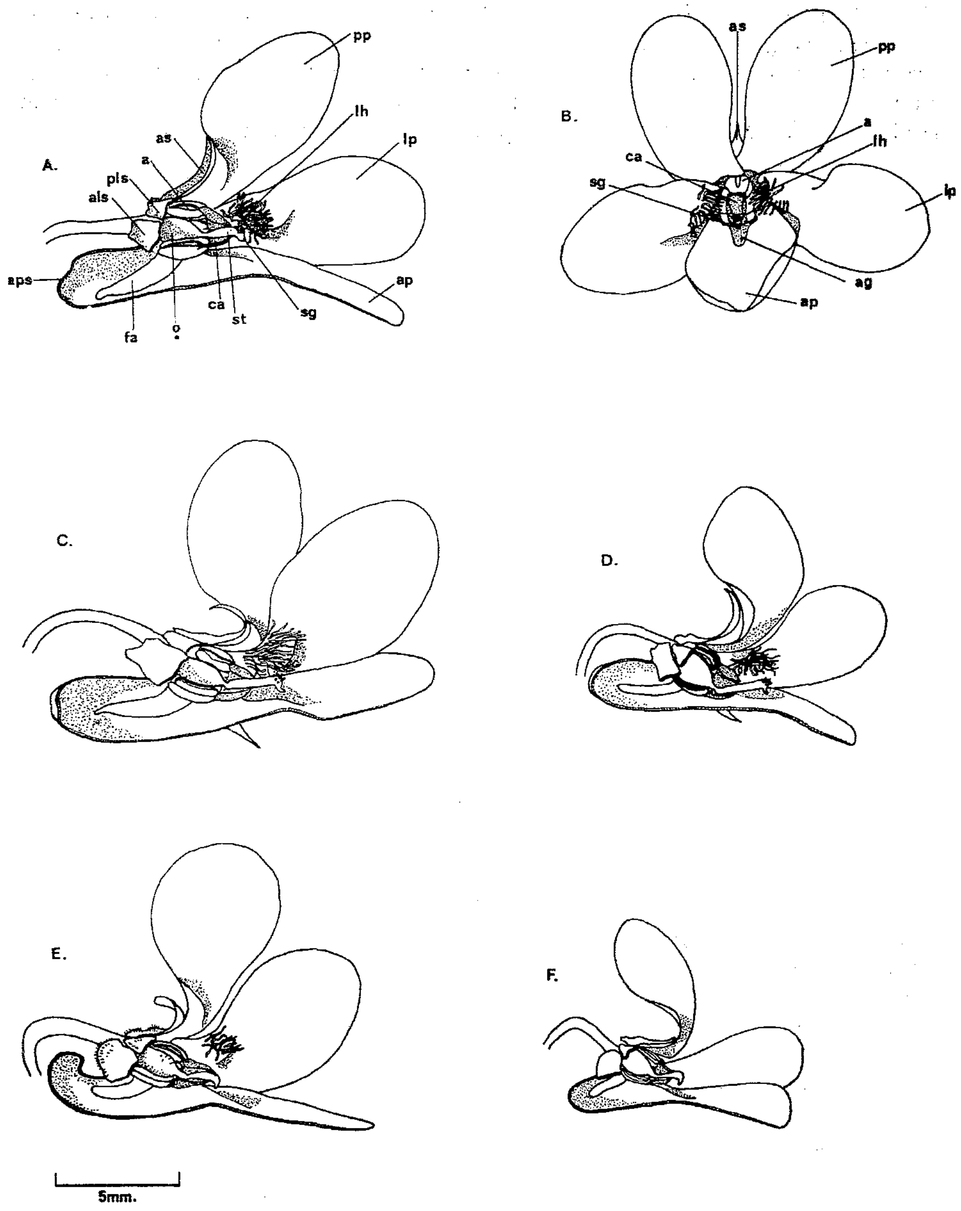

Fig. 1. Half-flower diagrams of A. *Viola reichenbachiana*, C. *V. riviniana* subsp. *riviniana*, D. *V. riviniana* subsp. *minor*, E. *V. hirta* subsp. *hirta*, F. *V. hirta* subsp. *calcarea* and B. a ventral or 'insect's eye view' of *V. reichenbachiana*.

Symbols: a = anther
ag = anterior groove
als = anterio-lateral sepal
ap = anterior petal
aps = anterior petal spur
as = posterior sepal
ca = connective appendage
fa = filament appendage
lh = lateral hairs
lp = lateral petal
o = ovary
pls = posterio-lateral sepal
pp = posterior petal
sg = stigma-opening
st = style.

of pollen either on the stylar papillae or within the stigmatic cavity. Of the five that did, two had been ravaged by snails, one was in a bag into which a pair of March flies (*Bibio ferruginatus* L.) had found their way, and another had been damaged by a deer. Signs of ovule development were present in two of these disturbed flowers. No foreign pollen was observed at all. These results strongly suggest that self-pollination is uncommon.

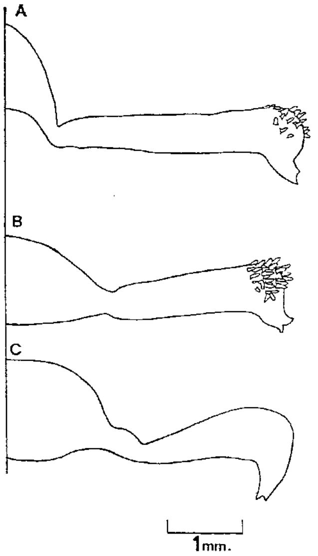

Fig. 2. Styles of A. *Viola riviniana*, B. *V. reichenbachiana*, C. *V. hirta.*

Emasculations

Thirty flowers were emasculated whilst still in bud and then allowed to bloom normally. After four days of warm, sunny weather they were gathered and placed immediately in preservative.

In the laboratory each flower was dissected and examined for the effectiveness of emasculation; only two specimens bore traces of anther tissue. The styles were mounted in the usual way and examined for pollen. The ovules were checked for signs of development.

Twenty-seven out of thirty styles contained *Viola* pollen, and thirteen ovaries contained developing ovules. In most specimens the pollen was deeply inserted into the stigmatic cavity but frequently there was sufficient to form a bunch of grains adhering to the stigma-opening. Little pollen was found in the papillary region of the style. The average number of violet pollen grains per stigmatic cavity was fifteen, and the average number of foreign grains (mostly of *Endymion non-scriptus*) was six (see Fig. 3). This is positive evidence that transference of pollen is frequent under suitable conditions.

CONTENTS OF STIGMATIC CAVITIES

A total of 525 stigmatic cavities were examined. The pollen grains were clearly visible when stained with basic fuchsin but it was usually impossible to tell whether or not they were viable. Wrinkled and empty grains appeared in the samples very occasionally. Detection of pollen transfer between the species of *Viola* was not possible because of the difficulties in identifying the pollen grains. Occasionally very large numbers of pollen grains were found in a cluster at the stigma-opening. The capacity of the stigmatic cavity varied from specimen to specimen and so the number of grains they could contain also varied considerably. It is difficult to estimate how many grains filled a stigmatic cavity, but the figures from the present study suggest that the number must be about thirty for *V. riviniana* and *V. reichenbachiana*, and somewhat less for *V. hirta*.

The data are summarized in Table 2. The term 'blossom' is used here in the sense of Faegri & van der Pijl (1966), that is, as an ecological term as opposed to a morphological one. 'Shelter' denotes the shade afforded by the dominant trees or shrubs.

The occurrence of large numbers of foreign pollen grains in some stigmatic cavities was most striking. In view of the limited capacity of the stigmatic cavity it would be expected that homogeneity of pollen loads would be at a premium. The presence of foreign grains suggests the strong competition for pollen vectors amongst the spring-flowering plants.

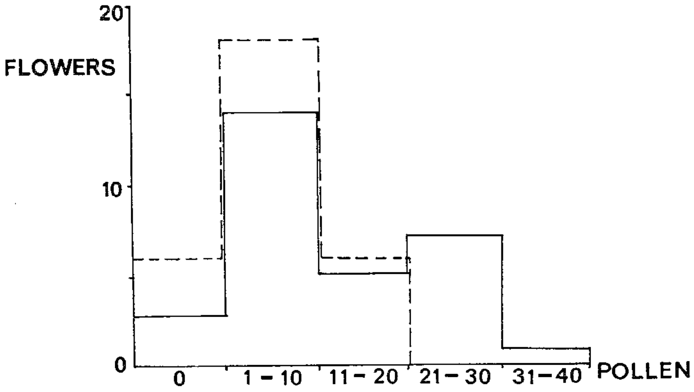

Fig. 3. Histogram of the number of pollen grains per stigmatic cavity in emasculated flowers. Ordinate: number of emasculated flowers, abscissa: absolute counts of pollen in stigmatic cavity. Solid line indicates *Viola* pollen; broken line indicates *Endymion* pollen.

EFFECTS OF WEATHER AND SHELTER

The influence of the weather preceding the collection of flowers of *V. hirta* is illustrated by Fig. 4. Comparative data for *V. reichenbachiana* show a large decrease in the number of pollen grains in the stigmatic cavities after rain, although the percentage of specimens containing them remains remarkably constant in all weathers (samples 6, 7, 8). The effect on *V. hirta* is very striking, and as all samples of this species were taken from exposed habitats the combined influence of weather and absence of shelter may be inferred from these results. Rain is detrimental and sometimes disastrous to insects trapped in the open whilst visiting flowers (Perkins 1919, Rau 1935), and Rayment (1935) has described how rain can occasionally lead to the decapitation of solitary bees. It is to be expected that wind and rain will eliminate insect visitors more rapidly in exposed places than in sheltered ones. My own observations confirm that some visitors to *Viola* (*e.g.* the syrphid *Platychirus albimanus* Fabr. and the bumblebee *Bombus agrorum* Fabr.) will remain active even in very light rain when sheltered by a canopy of tree branches, but not in exposed places. Also they recommence activity sooner after bad weather in sheltered habitats. Again Frison (1923) pointed out that the first bees out in spring are essentially bees which nest in the woods where the blossom at the time of their emergence is located. It is interesting thus to note that the percentage of *Viola* pollen per sample in *V. reichenbachiana* differs only marginally between sheltered and exposed habitats.

Viola riviniana samples reveal a different situation. The percentage of *Viola* pollen drops markedly at habitat B (edge of wood), which was sheltered, and remains consistently

TABLE 2. Data on contents of stigmatic cavities. Symbols for habitat as in Table 1. Symbols for preceding weather show type of weather followed by number of days of this type. Symbols for shelter: + presence or — absence. Symbols for foreign pollen: E = *Endymion non-scriptus*, A = *Anemone nemorosa*, P = *Primula vulgaris*, T = *Taraxacum officinale*, Gl = *Glechoma hederacea*.

Species	Sample		Percentage of sample containing			Total number of pollen grains per sample		Percentage of pollen total		Habitat	Corolla	Preceding weather	Shelter	Other Blossoms	Foreign pollen			
	No.	Size	*Viola* only	*Viola* & Foreign	No pollen	*Viola*	Foreign	*Viola*	Foreign						E.	A.	P.	T.
V. riviniana	11	30	76·6	3·4	20·0	414	2	99·8	0·2	F	large	sun 5	—	many	+	+		
	12	30	66·7	20·0	13·3	358	16	95·7	4·3	F	typical	sun 5	—	many	+			
	13	15	40·0	13·3	46·7	61	4	93·8	6·2	B	typical	sun 5	+	few	+			+
	14	30	17·0	46·6	36·4	—	—	—	—	B	typical	sun 1	+	many (1966)	+	+		
subsp. *minor*	16	30	83·3	0	16·7	510	0	100	0	H	small	mixed	—	none				
	17	30	93·3	0	6·7	470	0	100	0	H	typical	mixed	—	none				
V. reichenbachiana	6	18	72·2	11·0	16·8	226	9	96·2	3·8	E	typical	rain 2	+	few	+		+	
	7	30	60·0	30·0	10·0	462	21	95·7	4·3	E	typical	sun 5	+	few	+			
	8	30	70·0	23·3	6·7	382	28	93·2	6·8	F	typical	sun 5	—	many	+	+	+	
	9	30	90·0	0	10·0	585	0	100	0	A	typical	sun 5	+	none				
	10	30	66·7	23·3	10·0	419	24	94·6	5·4	D	typical	sun 5	—	many	+		+	
V. hirta	1	15	13·3	0	86·7	3	0	100	0	C	mixed colours	rain 2	—	few				
	2	30	50·0	3·3	46·7	119	1	99·2	0·8	C	mixed colours	sun 5	—	few				+
	3	30	38·5	3·9	57·6	93	1	99·4	0·6	D	pale	sun 2	—	many				
	4	30	63·3	0	36·7	162	0	100	0	D	dark	sun 3	—	many				
	5	30	73·3	3·3	23·4	167	4	97·7	2·3	D	mixed colours	sun 5	—	many				Gl
subsp. *calcarea*	18	30	42·9	0	57·1	128	0	100	0	H	pale	mixed	—	none				
	19	7	65·0	0	35·0	22	0	100	0	H	small pale	mixed	—	none				
	20	30	85·0	0	15·0	154	0	100	0	H	dark	mixed	—	none				
V. odorata	15	20	65·0	0	35·0	125	0	100	0	G	white	sun	+	none				

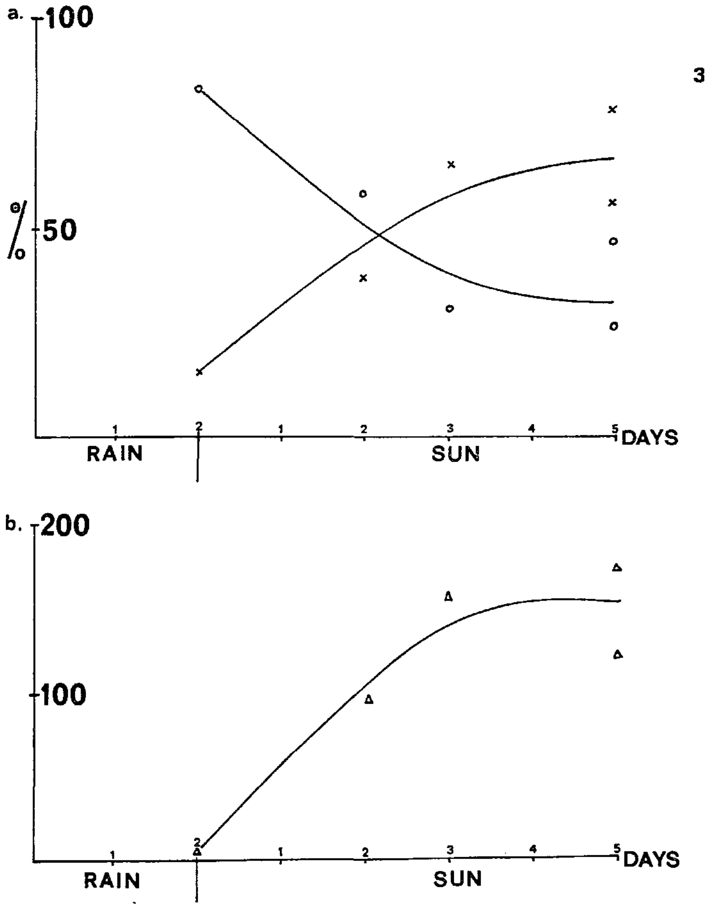

Fig. 4(a). Percentage pollination of samples of *Viola hirta* plotted against the number of days of rain or sun preceding the collection of the samples, x = percentage of sample containing *Viola* pollen only, o = percentage of sample containing no pollen.

(b) Actual counts of *Viola* pollen grains in samples of *V. hirta* plotted against the number of days of rain or sun preceding the collection of samples.

higher in the open habitats. This point will be met subsequently when the presence of other blossoms is discussed, but it is worth mentioning here that this habitat was more deeply shaded than the others, being further into the wood. This may well have discouraged some insect visitors; Kerner & Oliver (1902) reported that deep shade of this nature excludes many insects.

Pollination varies widely after unsettled weather and this may be expected from the differential responses of insect groups to changes in weather. Linsley (1958) notes that bumble bees (*Bombus* spp.), will remain active in cloudy weather while many solitary bees (*e.g. Andrena* spp.), will cease activity directly cloud obscures the sun; females return to the nest and males seek some sheltered spot. Again Perkins (1919) noted that solitary bees never leave their nests unless the sun is shining, and that they appear to be aware of imminent changes in the weather, ceasing activity as soon as the sun disappears behind cloud.

EFFECTS OF DIRECTION OF SLOPE AND TEMPERATURE

Fig. 5 presents data which strongly suggests that the direction of slope affects pollination. Slope and shelter together control the amount of sunlight incident upon the actual habitat occupied by the violets. As indicated above a large variety of insect visitors to *Viola* are strongly affected by the presence or absence of sunlight and many require a minimum temperature before commencing activity. The behaviour of many of the dipteran visitors is strongly influenced by the temperature, which must rise above a flight-threshold (Knight 1967). The emergence of many solitary bees is associated with the temperature of the ground in which they are overwintering. Shading of the burrow entrances, for example by vegetation obscuring the sun as it moves in its arc, may inhibit activity entirely (Linsley 1958). It is to be expected, therefore, that west and south-facing slopes will foster more potential visitors than north and east-facing slopes, and flight into sheltered habitats will be less common until temperatures there remain at a higher level.

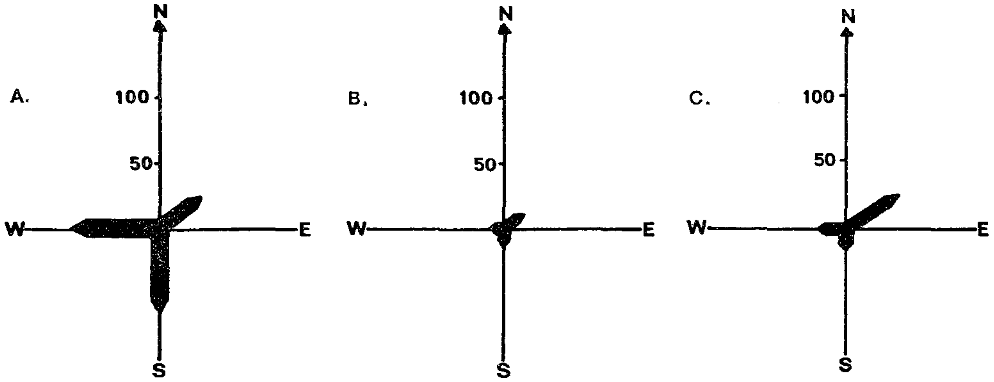

Fig. 5. Diagrams to show the relationship between percentage pollination and the direction of slope of the habitat. Habitat types are north, east, south or west facing slope.

A. Average percentage with *Viola* pollen only, per habitat type.

B. Average percentage with *Viola* + foreign pollen, per habitat type.

C. Average percentage with no pollen, per habitat type.

EFFECTS OF PROXIMITY OF BLOSSOMS OF OTHER GENERA

Table 2 shows that pollen of four species, besides *Viola*, was found in the stigmatic cavities: *Endymion non-scriptus*, *Primula vulgaris*, *Anemone nemorosa* and *Taraxacum officinale*. Although the actual numbers per sample are extremely small when compared to the numbers of *Viola* grains, it is clear that the more blossoms of other species there are in a habitat, the more foreign pollen is deposited in the stigmatic cavities of violets, see Fig. 6b; also, the percentage containing *Viola* and foreign pollen together remains highest in these habitats, see Fig. 6c. The *Viola* pollen counts were very similar from habitats in which there were no blossoms of other genera and those with many; for example all four species of foreign pollen were recorded from habitat F (coppiced wood), yet the count remained high. However, in habitat B (edge of wood), the *Viola* pollen count dropped markedly and in 1967 the number of blossoms of other species was scant. It seems, therefore, that the samples can be categorized according to the frequency of other blossoms in the habitat from which they were collected, namely: none, few and many (see Fig. 6a, b, c).

In the first category, anthophilous insects (those feeding on flowers) seeking food must visit *Viola*, consequently the incidence of pollen in the stigmatic cavities is high. In the third category violets may actually benefit from the presence of other blossoms: data from

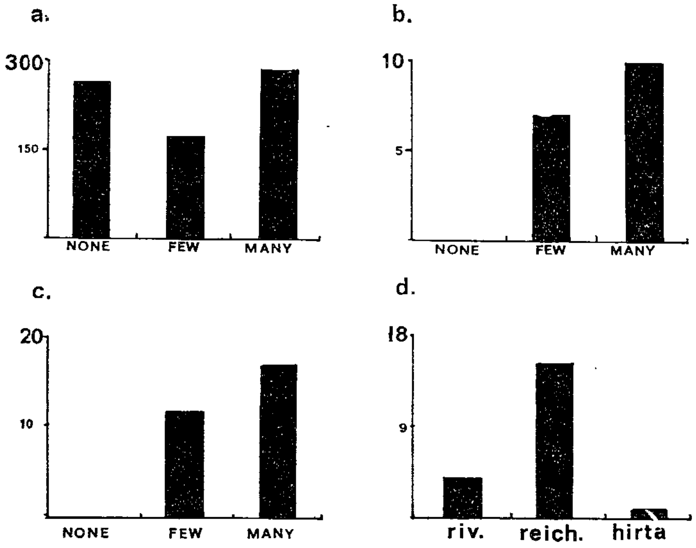

Fig. 6a, b, c. Diagrams to show the relationships between pollen counts and habitat type; habitat types are: None = no blossoms of other genera, Few = few blossoms of other genera, Many = many blossoms of other genera. (a) Average *Viola* pollen counts, (b) average foreign pollen counts, and (c) average percentage with both *Viola* and foreign pollen.

d. Average foreign pollen count for each species of *Viola;* riv. = *V. riviniana*, reich. = *V. reichenbachiana*, hirta = *V. hirta.*

visitor collections in these three habitat types show that those with a variety and abundance of conspicuous blossoms enjoy the greatest number and variety of insect-visitors. By examining the pollen carried by these insects it has been possible to show that a proportion of them visit *Viola* although probably initially attracted to the spot by more conspicuous blossoms. Also, while the number of foreign pollen grains in the stigmatic cavities is increased, pollination is maintained at a high level. That insect visits are increased by other blossoms is suggested also by the peak for *V. reichenbachiana* in Fig. 6d. This species had more visits from Diptera, less constant to one blossom, than the other two violet species. It may be prudent to point out here that there is no evidence from my own studies to suggest that the presence of foreign pollen in a stigmatic cavity interferes with the growth of *Viola* pollen-tubes.

In the second category (few other blossoms), percentage pollination fell, particularly in habitat B in 1966. Data from analyses of pollen on insect visitors to *Viola* show *Endymion* to be the most frequent foreign pollen found mixed with *Viola*, also *Endymion* is the most commonly occurring contaminant in the present study. In habitat B there were large stands of *Endymion* in 1966. The figures suggest, firstly, that some visitors to *Viola* also frequently visit *Endymion*, and secondly, that the flowers of the latter may be the most attractive to these visitors hence tending to monopolize their attentions and bringing about a fall in the pollination of *Viola*.

Another blossom present in great abundance in some habitats was *Primula vulgaris*, but very little pollen of this species was found in the samples. Detailed observations in the field established that the most frequent, and almost sole diurnal visitors to *Primula* in these habitats were the bee-fly *Bombylius major* L. and the Brimstone butterfly *Gonepteryx rhamni* L. and they were constant to this blossom. There were, therefore, no vectors evident that carried *Primula* pollen to *Viola* during daylight. However, although in the course of a week of observations all three *Viola* species were seen to receive visits from *Bombylius major*, *Primula* pollen was found in *V. reichenbachiana* alone. Detailed analyses of insect visitors to *Primula vulgaris* and to the three species of *Viola* (to be published elsewhere) show that in some habitats *Primula vulgaris* and *V. reichenbachiana* are visited chiefly by Diptera whilst the remaining species of *Viola* receive visits from a wider variety of insects, especially Hymenoptera. The present work suggests that *Primula vulgaris* and *V. reichenbachiana* have the most visitors in common and reveals the activity of individual specimens that visited them both.

V. hirta differs from the other two *Viola* species in that it flowers earlier than both *Endymion* and *Primula*. The only foreign pollen found in samples of this species was that of *Taraxacum*, and then in very small quantities (Fig. 6d). The proportion of stigmatic cavities of this species containing no pollen at all (Table 2) suggests that while attraction from more conspicuous blossoms is less of a problem than a month later, the flowering period is so early as to be in the critical period of fluctuating temperatures governing the emergence and flight of its visitors.

EFFECTS OF PROXIMITY OF BLOSSOMS OF THE SAME GENUS

Evidence on the effect of the proximity of other violets is very scant, but inspection of Table 2, samples 3, 18, 4 and 20, shows that pollination in pale and dark specimens of *V. hirta* does differ markedly; this point is illustrated by Fig. 7. Sample 15, from an isolated clump of white-flowered *V. odorata* gives a high figure for percentage pollination. This contrasts with those for pale corollas and suggests that in the company of dark violets pollination of pale ones falls off, but when growing in isolation (sample 15) pollination is maintained at a high level.

V. riviniana subsp. *minor* and *V. hirta* subsp. *calcarea* are both small-flowered violets and both maintained a high percentage pollination although subsp. *minor* contained more pollen per stigmatic cavity. There is very little evidence from these figures that corolla-size influences pollination in *Viola*. However, bumblebees attempting to enter them and evidently not succeeding in obtaining any food frequently fly off, or crawl over the petals, pierce the petal spur and suck the nectar through the hole. Brian (1957) has described how certain

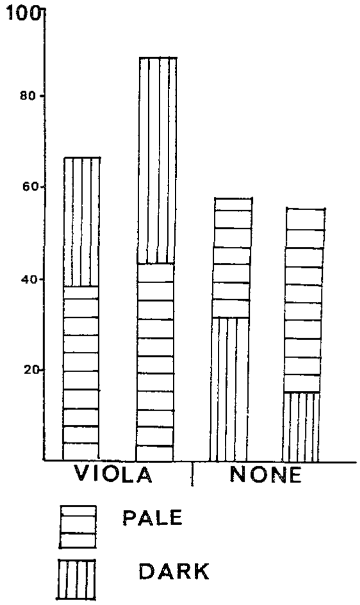

Fig. 7. Percentage pollination of pale blue and dark blue flowers of *Viola hirta*. Ordinate: percentage pollination, abscissa: left, samples containing *Viola* pollen only; right, samples containing no pollen.

species of bumblebees are prone to this 'thieving' behaviour, and it is possible that some flowers are not pollinated because of it.

Tetracolpate grains, at present thought to be confined to *V. riviniana*, were occasionally found in the stigmatic cavities of *V. reichenbachiana* and *V. hirta*. This is evidence that interspecific pollen transfer within the genus does occur, but quantitative estimates were not possible in this study.

DISCUSSION AND CONCLUSIONS

The question of the distinction between insect visitors and pollinators, and data on their pollen loads and behaviour, are being prepared for a subsequent paper. The present work is concerned only with the results of insect activity and the effects of weather, shading and other blossoms upon it. Rain can be harmful to insects caught visiting flowers and can reduce pollination in exposed places. Shaded or north or east facing habitats may experience similar reductions in pollination as a result of the diminution of sunlight incident upon the insects and their homes. Pollination is frequent in habitats in which *Viola* is the only available source of food for anthophilous insects. Habitats with a variety of blossoms may lure sufficient insects to ensure the pollination of all species including the inconspicuous ones. Large stands of showy blossoms may monopolize the insect visitors in habitats also occupied by less attractive plant species.

Stebbins (1959) in his review on the role of hybridization in evolution concludes that, in general, hybridization is increased in marginal and changing environments. More specifically Anderson (1954), working on populations of *Viola*, reported an increased degree of introgression in disturbed sites. Again Camp (1961) describes the rapid changes in gene frequency in violet populations growing in disturbed and marginal habitats, and points out that the genus *Viola* is at present going through an explosive phase in its evolution. This must be attributed partially to the character of the breeding system which in *Viola* combines regular in-breeding, chiefly by means of summer cleistogamous flowers, with occasional out-breeding followed the fertilization of chasmogamous flowers in spring (Valentine 1962). It has been evident in the course of my own work that disturbed and marginal habitats attract a greater number and variety of insects (see pp. 152–153) and it is these habitats that yielded samples containing the highest numbers of foreign pollen grains (Table 1, habitats, B, D, E, F). Deposition of foreign pollen in the stigmatic cavities of the chasmogamous flowers may be taken as proof of the transference of pollen between species and we have seen that this process was most frequent in the types of habitat under discussion. It seems reasonable to suppose that in these situations the out-breeding capability of *Viola* most nearly fulfills its potential.

Current theory on natural hybridization as outlined by Stebbins (1959) and substantiated by the work of a number of other authors (*e.g.* Anderson 1954) maintains that establishment of hybrids is more common in disturbed or marginal habitats than in stable or uniform ones. The results of the present work on *Viola* suggest that, in some entomophilous species at least, one of the reasons for this is that prior to establishment there exists a greater variety of genotypes available for selection as a result of a (temporary) shift to out-breeding. There is a marked increase in the pollination of the chasmogamous flowers, and in the production of seed from them. The overall effect is the release of a greater than normal variety of recombitants, some of which may increase the chances of exploiting these kinds of environment. That disturbed and marginal habitats may lead to a greater abundance and variety of pollinators, and that this factor strongly influences the frequency of hybridization, has not been fully appreciated in evolutionary studies.

ACKNOWLEDGMENTS

I wish to thank Professor C. D. K. Cook for his help and encouragement during the preparation of this paper. My thanks also to Professor H. G. Baker for scrutinizing the manuscript and for commenting upon it. I thank Mr. Sankey of the Juniper Hall Field

Centre for making available facilities for fieldwork and Sir Geoffrey and Lady Codrington for their most generous and prolonged hospitality whilst fieldwork was in progress. My thanks are due also to the University of Liverpool for the provision of a grant for this research.

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