STUDIES ON BRITISH PANSIES
I. CHROMOSOME NUMBERS AND POLLEN ASSEMBLAGES

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ABSTRACT

The chromosome counts and pollen assemblages of a range of British pansies are given. With the exception of what is believed to be an aberrant count for *Viola nana* Corb. (*V. kitaibeliana* in Dandy, 1958), the chromosome counts confirm those cited by past workers. The polymorphic pollen assemblages which can be characterized by the most abundant grain type and the associated subsidiary types show that the taxa can be divided into two main groups.

In the case of the two variable and often confused species, *V. tricolor* and *V. arvensis*, differences in their pollen assemblages confirm their separation on the basis of chromosome counts and such is the agreement between the two criteria that the former should be a reliable criterion for the separation of the two taxa.

1. INTRODUCTION

The *Melanium* subgenus of *Viola*, comprising those plants better known as pansies, forms a very variable group not readily divisible into coherent taxa. Past treatments of the complex of variation surrounding *V. tricolor* sensu lato have ranged from the acceptance of a few rather variable species to the erection of many, narrowly-defined microspecies. The cytogenetical studies on European pansies by Clausen (1921, 1922, 1924, 1926, 1927, 1931) and the cytological investigations of British plants by Fothergill (1944) have, however, done much to clarify and stabilize the species concept in the subgenus. The Jordanian microspecies recognized by Drabble (1909, 1926, 1927 *a-c*, 1928) were shown to be indistinguishable on the basis of chromosome number and chromosome morphology, although Drabble's major groupings of these—*Luteae*, *Curtisiae*, *Tricolores*, *Arvenses* and *Nanae*—could be characterized by such features. On this basis the *Tricolores* are not separable from the *Curtisiae*, or the *Luteae* from the *Nanae*. The latter pair are, nonetheless, readily divisible on morphological features.

As Clausen (1927, 1931) pointed out whilst discussing the concept of species within the subgenus *Melanium*, it is quite impossible to give a definition covering all cases. Morphological differences coupled with complete or partial sterility fail to delimit all taxa satisfactorily because there are some morphologically uniform taxa which contain several intersterile cytotypes. On the other hand, differences in chromosome number used as the only means of delimiting species also fail because of the limited association between morphological appearance and chromosome number. In the particular case of the *tricolor–arvensis* group of annuals, Clausen (1922) recommended the retention of two species, *V. tricolor* L. and *V. arvensis* Murr., as the basic units, mainly on the grounds that two different chromosome numbers were associated with two reasonably well-defined morphological types which had differing edaphic ranges. It is sometimes felt, however, that this division into two species is not always one easy to maintain, for their limits are blurred by a rather complex variation which itself may be further complicated by the reputedly frequent occurrence of hybridization and introgression of the two species.

During an investigation into the possibility of frequent interspecific hybridization between *V. tricolor* and *V. arvensis* a considerable number of observations were made on the cytology and pollen morphology of a representative range of British pansies. Since these observations emphasized the distinctness of the two taxa within the British Isles

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and are pertinent to the problem of hybridization to be discussed in detail elsewhere, they form the basis of the present paper.

There is, perhaps, little need to stress the value of cytology in understanding the complex variation in the pansies, but the potential value of pollen morphology appears relatively unknown.

Wittrock (1897) was the first to describe the quantitative differences in the polymorphic pollen assemblages of *V. tricolor* and *V. arvensis* and to use them as an additional character in discriminating between the two taxa. These differences were subsequently mentioned by Clausen (1922) although he made little use of the character in his final analysis of the taxa. Since then the taxonomic value of the character has been overlooked with the one recent exception when Mullenders & Mullenders (1957) used it as a criterion for deciding whether *V. maritima* Schweigg. (= *V. curtisii* Forst., *V. sabulosa* Bor.) should be considered as a species distinct from *V. tricolor* subsp. *tricolor*.

Before detailing the observations on the cytology and pollen morphology of this group of plants—where there has been considerable difference of opinion over specific limits in the past—it would be wise to define the limits of taxa used in this account. With the exception of certain modifications in application the specific names used here largely follow the usage of Warburg (1952) as the observations were originally made within this framework. The main exception concerns the *tricolor–arvensis* complex where much of the work was concentrated and where the three groups of plants were distinguished as follows:

V. tricolor: Flowers blue, purple or predominantly so; upper petals longer than sepals (usually more than $\times 1\frac{1}{2}$); stylar flap large and relatively conspicuous.

V. arvensis: Flowers all cream or occasionally with upper petals tipped with blue-purple; upper petals shorter than, or sometimes as long as, the upper sepals; stylar flap so reduced as to be, or appear to be, absent.

Intermediates between V. tricolor and V. arvensis (subsequently abbreviated to 'AT-intermediates'): Flowers cream, or predominantly so; upper petals longer than upper sepals (usually $\times 1\frac{1}{4}$–$1\frac{1}{2}$); stylar flap present but approximately intermediate in size between that of *V. arvensis* and *V. tricolor*.

The AT-intermediates formed a rather arbitrary group which was meant to cover all those plants not readily ascribable to *V. tricolor* and *V. arvensis*, and which might be considered to be putative hybrids. Observations on these and experimentally-produced hybrids between *V. tricolor* and *V. arvensis* will be the subject of a following paper.

It will be seen that *V. tricolor*, as here defined, is more or less equivalent to *V. tricolor* subsp. *tricolor* of Warburg (1952); in consequence, *V. tricolor* subsp. *curtisii* of the latter is here referred to as *V. curtisii*.

2. Chromosome counts

Previous chromosome counts for taxa represented in the British Isles made on British and other European pansies by Clausen (1921, 1926, 1927, 1931) and Fothergill (1944) may be summarized as :

V. lutea	$2n = 48$
V. curtisii	$2n = 26$
V. tricolor	$2n = 26$
V. arvensis	$2n = 34$
V. nana	$2n = 48$

It would thus appear that chromosome number offers an important criterion for the separation of *V. arvensis* from *V. tricolor*, and *V. arvensis* from the *V. kitaibeliana* complex (includes *V. nana*), two pairs of taxa difficult to delimit satisfactorily on purely morphological criteria. Counts reported by Fothergill (1944) for *V. variata* var. *sulphurea* ($2n = 26$, $2n = 28$) and *V. contempta* ($2n = 40$) refer to AT-intermediates as defined above and will be discussed in another paper.

TABLE 1. List of chromosome counts.

	Reference Number of Population	*Locality*	*Chromosome number* PMC (*n*)	RT (2*n*)
lutea	L18	Moorhouse, Westmorland	24	—
	L27	Malham Tarn, Yorkshire	24	—
	L28	Combs Dale, Derbyshire	24	—
	L50	Widdybank Fell, Teesdale, Co. Durham	24	—
	L52	Schiehallion, Perthshire	24	—
	L58	Wardlow Hay Cop, Derbyshire	24	—
curtisii	TC44	Aberffraw, Anglesey.	13	—
	TC67	Kenfig Burrows, Glamorgan	13	—
	TC68	Broughton Burrows, Gower, Glamorgan	13	—
	TC69	Llangennith Burrows, Gower, Glamorgan	13	—
	TC81	Castlegregory, Co. Kerry, Ireland	13	—
	TC83	Corbally, Co. Waterford, Ireland	13	—
tricolor	T10	nr. Aberystwyth, Cardiganshire	13	26
	T14	nr. Durham, Co. Durham	13	26
	T20	Orpington, Kent	13	—
	T23	nr. Aberfeldy, Perthshire	13	—
	T25	Lawers Burn, Perthshire	13	—
	T34	Feltham, Middx.	13	—
	T79	High Force, Teesdale, Co. Durham	13	—
	T80	Yetholm, Roxbs.	13	—
arvensis	A1	Southampton Common, Hampshire	17	—
	A2	Highfield, Southampton, Hampshire	17	—
	A5	Swaythling, Southampton, Hampshire	17	—
	A6	Sholden Downs, nr. Deal, Kent	17	—
	A7(A)	Kingsdown, nr. Deal, Kent	17	34
	A10	nr. Aberystwyth, Cardiganshire	17	34
	A11	nr. Aberystwyth, Cardiganshire	17	—
	A12	nr. Durham, Co. Durham	17	—
	A21	Frome, Somerset	17	—
	A22	Porton, nr. Salisbury, Wiltshire	17	34
	A26	nr. Southampton, Hampshire	17	34
	A29	Long Down, New Forest, Hampshire	17	—
	A32	Kempshott, nr. Basingstoke, Hampshire	17	34
	A35	Stoborough, nr. Wareham, Dorset	17	—
	A36	nr. Hursley, Hampshire	17	—
	A37	nr. Hursley, Hampshire	17	—
	A38	nr. Rockland Broad, Norfolk	17	—
	A47	nr. Perranporth, Cornwall	17	—
	A48	St. Martin's, Isles of Scilly	17	—
	A49	St. Mary's, Isles of Scilly	17	—
	A55	Chilworth, nr. Southampton, Hampshire	17	—
	A59	Twyford, nr. Winchester, Hampshire	17	—
	A61	Arne, Dorset	17	—
	A66	St. Mary's, Isles of Scilly	17	—
	A70	nr. Eastleigh, Hampshire	17	—
	A71	nr. Romsey, Hampshire	17	—
	A72	nr. King's Somborne, Hampshire	17	—
	A75	nr. Corhampton, Hampshire	17	—
nana	N45	St. Brelade's Bay, Jersey	24	—
	N46	St. Ouen's Bay, Jersey	24	—
	N64	Tresco, Isles of Scilly	24	—
	N65	Bryher, Isles of Scilly	24	—

(a) *Methods*

For reasons of practical convenience most chromosome determinations were made from squash preparations of PMC meiosis although confirmatory observations were sometimes made on similar preparations of root-tip mitosis.

In the latter case root-tips were pretreated for approximately four hours with a saturated solution of 8-hydroxyquinoline before fixation in 1:3 acetic alcohol and stained with acetic-orcein as in the technique of Tjio & Levan (1950).

When PMC meiosis was used satisfactory stages in division were found when the flower buds were *c.* 2 mm long and almost invariably occurred during the late morning. Flower buds were fixed in Carnoy's solution for approximately ½ hour and then were transferred to 1:3 acetic-alcohol, containing a little ferric acetate or chloride, and were usually stored at −15° C in this solution until needed. The PMCs were subsequently stained with acetocarmine solution. Observations were usually made on temporary slides ringed with a rubber solution to avoid the loss of contrast in staining sometimes experienced when the slides were being made permanent by the dry-ice method (Conger & Fairchild 1953).

In most cases counts were made from several cells on each slide and, wherever possible, repeated on 1–5 plants in each population examined. Whenever a population was morphologically variable, counts were also made from as many of the main types as possible.

(b) *Observations*

Details of the counts obtained are given in Table 1 and illustrated in Figs. 1 and 2. With but a single exception they agree with those already listed. The exception concerns a single plant of *V. nana* from six originally collected from Tresco, Isles of Scilly, and transplanted to the gardens at Southampton University. This plant had $2n = 50$, instead of $2n = 48$ as in the other five examples from the same population. The count was repeated on three different buds from the same plant and in all of these meiosis appeared perfectly

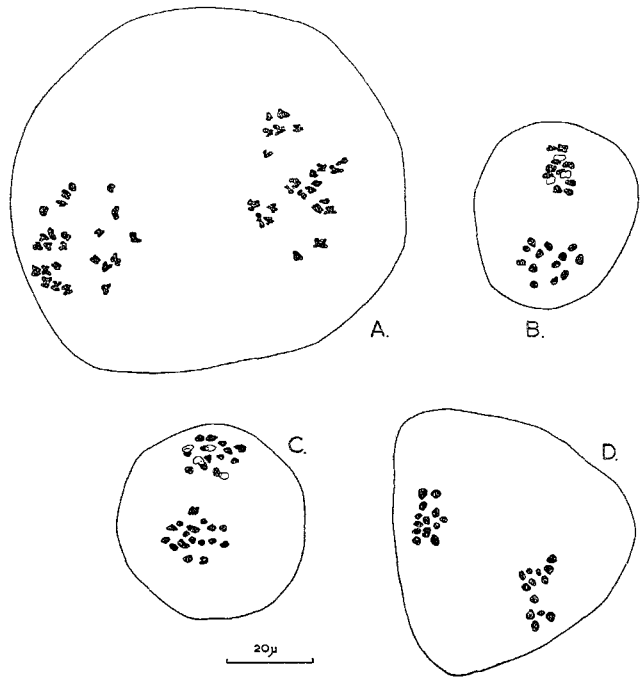

Fig. 1. Meiosis in PMCs. (a) *V. lutea* (L50): Prometaphase of second division, $n = 24$; (b) *V. tricolor* (T14): Second metaphase, $n = 13$; (c) *V. arvensis* (A75): Late stages of first anaphase, $n = 17$; (d) *V. curtisii* (TC44): Late stages of first anaphase, $n = 13$.

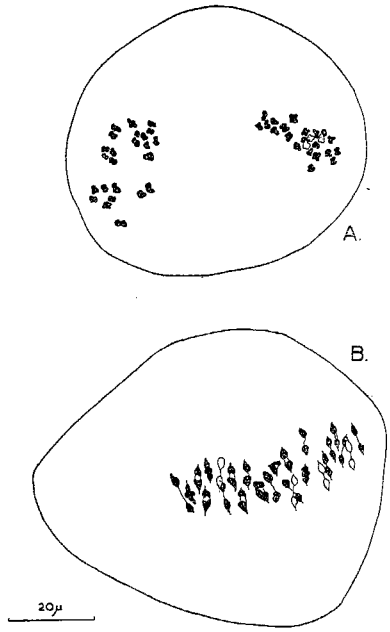

Fig. 2. Meiosis in PMCs of *V. nana* (N64). (a) Second metaphase, $n = 24$; (b) First metaphase with 25 bivalents, $n = 25$.

regular with the formation of 25 bivalents and without the expected occurrence of unpaired chromosomes or chromatid bridges. Since this particular plant was morphologically indistinguishable from others having chromosome numbers typical of *V. nana* and the count could not be repeated in other plants raised from seed collected from the original population, it was probably of little significance, being merely an aberrant chromosomal form which may have arisen as a result of primary nondisjunction of chromosomes or misdivision of a centromere in the previous generation.

Incidental observations on chromosome behaviour at meiosis have indicated some differences between the taxa studied. In many plants of *V. arvensis* 0·5–1·0% of the PMCs had two (or four) univalents which had failed to pair and were either lagging on the metaphase plate or lying disorientated in the cytoplasm (Fig. 3). Chromatid bridges were recorded only twice in this species. In plants of *V. nana* the only irregularities observed were one or two chromatid bridges at first anaphase in many PMCs of a single plant of N65; otherwise meiosis was perfectly regular. In this connection it is interesting to note that Clausen (1931), describing material of this taxon from Jersey, also observed 'slight irregularities' although he stresses that the plants had a 'fairly constant chromosome number'. He gave, however, no indication of the nature of these irregularities or how constant the chromosome number was.

In *V. tricolor* the frequency of chromosomal irregularity varied considerably from population to population. Although most plants examined had regular meiosis, others showed well-defined irregularities. Dicentric bridges and acentric fragments, suggesting the presence of large inversions, were observed in nearly all plants examined from T10 (Fig. 3). In both T25 and T79 some plants had chromatid bridges visible at first anaphase in most PMCs but poor staining made it difficult to be sure of their cause. As in *V. arvensis*, disorientated univalents were rare, being found with a similar frequency.

The situation in both *V. lutea* and *V. curtisii* was roughly parallel to that in *V. tricolor*. Plants from the majority of populations had regular meiosis whilst others in certain populations, notably L50 and L58 (*V. lutea*) and TC44 (*V. curtisii*) had high frequencies of chromatid bridges.

These differences in frequency of chromosomal irregularities might be associated with the breeding system of the taxa involved. Thus, meiosis in the predominantly inbreeding plants of *V. arvensis* and *V. nana* was most nearly always regular, whilst in the outbreeding plants of *V. tricolor*, *V. lutea* and *V. curtisii* chromosomal irregularities were more frequently observed. Presumably the nearly complete genetic homozygosity of the inbreeders would be responsible for their meiotic regularity, whereas the greater chromosomal heterozygosity in the outbreeders was responsible for their chromosomal irregularities.

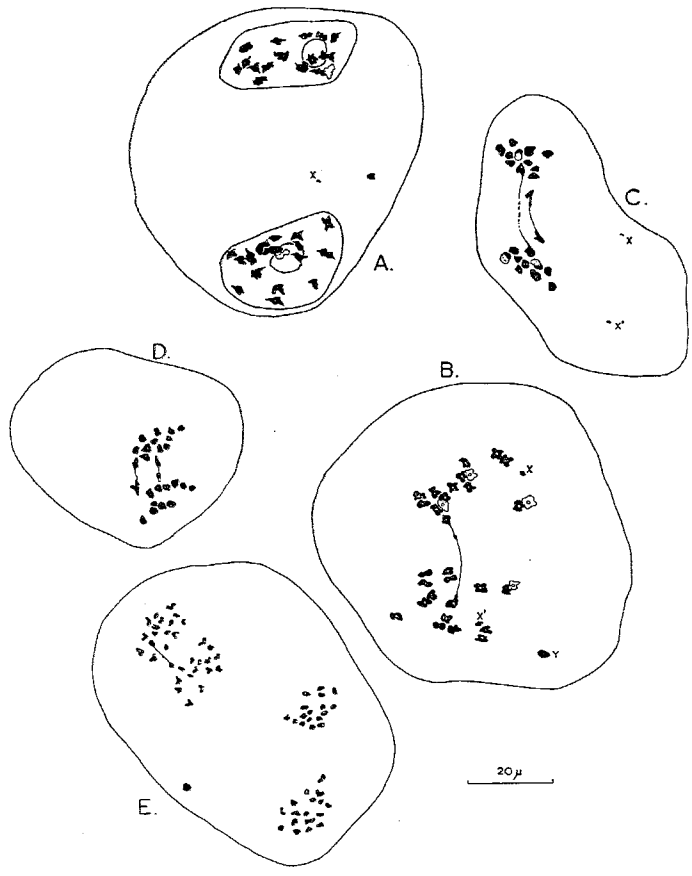

Fig. 3. Some irregularities in PMC meiosis. (a) *V. arvensis* (A36): Second prophase with univalent and fragment (X) in cytoplasm; the upper complement contains 16 chromosomes, the lower 17 chromosomes. $2n = 34$. (b) *V. arvensis* (A36): Prometaphase of second division with chromatid bridge persisting from first division; disorientated univalent (Y) from first division to one side; each complement has 17 chromosomes but the number has been maintained by the precocious division of the other univalent (X and X^1). $2n = 34$. (c) *V. tricolor* (T10): Late stages of first anaphase with two chromatid bridges and two acentric fragments (X and X^1). $2n = 26$. (d) *V. curtisii* (TC44): First anaphase with two chromatid bridges. $2n = 26$. (e) *V. arvensis* (A37): Second anaphase with a chromatid bridge between two chromosomes of upper complement; a univalent from the first division lying disorientated in cytoplasm; upper group of cells with 16 pairs of chromosomes, lower with 17 pairs of chromosomes.

3. Pollen Polymorphism

Specific references to the pollen morphology of *V. tricolor* and its immediate allies contain a number of confusing statements. Faegri & Iversen (1950) place *V. tricolor* into their group, the Stephano-colporatae, i.e. pollen characterized by four furrows with median pores. Wittrock (1897), comparing pollen of different species of pansies, stated that *V.*

tricolor had mainly 4-colpate grains with some 3- and 5-colpate grains, whereas *V. arvensis* had mainly 5-colpate grains with a few 4-colpate ones. On the other hand, data for these same two taxa given later by Erdtman (1952) would suggest they are not so readily distinguishable since both are recorded as having assemblages of 4-colpate grains, but with the occasional 5-colpate grains in *V. arvensis*. Counts given by Mullenders & Mullenders (1957) show *V. tricolor* subsp. *tricolor* with mostly 5-colpate and a few 4- and 6-colpate grains, and *V. maritima* Schweigg. (= *V. curtisii* Forst., *V. sabulosa* Bor.) with mostly 4-colpate, some 5-colpate and a few 3-colpate grains.

(a) *Methods*

Wherever possible, slides were made of a small sample of plants from each population with pollen from one flower of each representative plant being mounted on a slide. For good staining and ease of mounting a slight modification of the methylene-green/glycerine-jelly method of preparing pollen grains (Wodehouse 1935) was used.

Pollen grains were first stained on the slide with a weak solution of eosin in absolute alcohol and this was allowed to evaporate almost to dryness before the grains were mixed in a warm solution of methylene green in glycerine jelly and then covered with a coverslip. As the grains expanded fully before the jelly set, their furrow configuration was immediately obvious.

Pollen from both fresh and dried flowers was treated in this manner although pollen from the former was the most convenient to handle since the anthers had merely to be tapped on the slide to release the pollen. Anthers from herbarium specimens of less than a year's standing could be similarly treated after their dissection from the flower since the pollen still expanded quite readily in the glycerine jelly. Pollen from older or badly prepared herbarium specimens did not expand so readily, so the technique was slightly altered. The flowers were dissected to expose the anthers and then left overnight in an humid atmosphere to allow the grains to swell before the staining and mounting. Such material gave slightly inferior preparations but there was usually little difficulty in identifying the different pollen types.

The pollen assemblage of each plant was assessed by counting the different grain types on the slide. Both fully developed and aborted grains were counted over the entire field under the coverslip to avoid possible bias due to the differential movement between the larger, fully-developed grains and the smaller, aborted ones through the warm glycerine jelly on mounting. This usually entailed the counting of 300–1,200 grains.

(b) *Observations*

The counts for all plants of the populations studied are summarized in Table 2 and Fig. 4, which give the frequencies of the different pollen grains as mean percentages for the population. More detailed data for the individual slides are available in Pettet (1960).

These counts showed that the taxa represented by these populations could be divided into two groups according to the most abundant grain type and the less frequent types associated with it:

(i) *V. lutea*, *V. curtisii* and *V. tricolor*: mostly 4-colpate grains, with some 5- and a few 3-colpate grains.

Although 6-colpate grains normally did not occur in this group, a few examples were found in several plants from L50 (*V. lutea*) and TC68 (*V. curtisii*). In all cases their frequencies were very low, being of the order of 1 per 200–300 grains, and their appearances were of abnormal or unreduced pollen. They were larger than normal grains and had thickened exines and often poorly developed colpae. Two exceptional assemblages were also found. One plant in L50 had 71% of its assemblage composed of 5-colpate grains (cf. second group below), whilst another plant of L58 (*V. lutea*) had a greater proportion of 3-colpate grains than of 4-colpate ones.

TABLE 2. Pollen Assemblages of various populations

	Reference number of population	Number of Individuals	Mean percentage frequency of pollen grain types				Pollen abortion		Micrograins	
			3-colpate	4-colpate	5-colpate	6-colpate	Mean percentage	Range in percentages	Mean percentage	Range in percentages
lutea	L50	22	2·55	89·85	7·55	0·04	7·7	1·9–27·7	0·6	0·0– 1·9
	L58	10	7·64	90·53	1·84	—	26·6	7·8–49·9	1·9	0·0–12·6
curtisii	TC44	22	0·43	82·77	16·74	0·06	34·8	4·9–65·5	0·5	0·0– 1·7
	TC67	15	3·68	89·49	6·83	—	17·4	5·9–41·4	0·3	0·0– 0·9
	TC68	10	0·07	82·58	17·32	0·03	9·1	3·8–14·9	0·4	0·0– 0·9
	TC81	2	0·06	97·40	2·54	—	3·1	2·3– 3·9	0·7	0·7– 0·7
tricolor	T10	18	0·43	90·92	8·65	—	17·7	3·1–47·4	0·4	0·0– 1·8
	T14	2	—	81·35	18·65	—	2·8	2·2– 3·4	No information	
	T25	23	0·48	91·37	8·15	—	3·3	0·9–20·1	0·2	0·0– 0·9
	T80	10	0·13	95·92	3·96	—	18·8	3·8–47·7	0·6	0·0– 2·1
arvensis	A2	20	—	11·53	86·41	2·06	8·2	0·0–39·3	0·7	0·0– 7·4
	A7(A)	18	—	7·70	91·85	0·35	4·4	0·0–26·5	0·1	0·0– 0·3
	A10	10	—	9·87	89·70	0·43	2·3	0·5– 5·8	No information	
	A10(A)	4	—	0·07	68·89	31·04	2·2	0·9– 3·3	No information	
	A26	7	—	6·02	91·88	2·10	3·8	0·5–16·1	No information	
	A29	10	—	5·93	89·21	4·86	2·1	0·0– 5·6	0·3	0·0– 1·9
	A33	4	—	3·13	95·81	1·06	1·0	0·4– 2·1	0·1	0·0– 0·4
	A36	10	—	5·39	94·04	0·57	4·9	0·0–11·8	No information	
	A37	9	—	9·66	89·82	0·52	1·4	0·0– 3·4	No information	
	A42	2	—	26·86	72·19	0·95	2·8	2·6– 3·0	0·3	0·0– 0·5
	A66	9	—	0·77	91·37	7·80	1·9	0·7– 4·1	0·2	0·0– 0·8
nana	N45	9	—	8·63	87·45	3·92	48·5	32·8–63·1	0·1	0·0– 0·8
	N64	12	—	25·21	74·50	0·29	2·3	0·0– 5·7	0·1	0·0– 0·6
	N65	12	—	13·61	86·04	0·35	3·5	0·0–13·4	0·1	0·0– 0·7

(ii) *V. arvensis* and *V. nana*: mostly 5-colpate grains, with some 4- and 6-colpate grains. Tri-colpate grains were never recorded in this group but the frequency of the 6-colpate grains suggests that the latter correspond to them here. The 4-colpate grains were normally more frequent than the 6-colpate grains except in A10(A) (*V. arvensis*) where the latter were found in consistently higher percentages. A single plant with an exceptional assemblage resembling that of group (i) was recorded in N64 (*V. nana*).

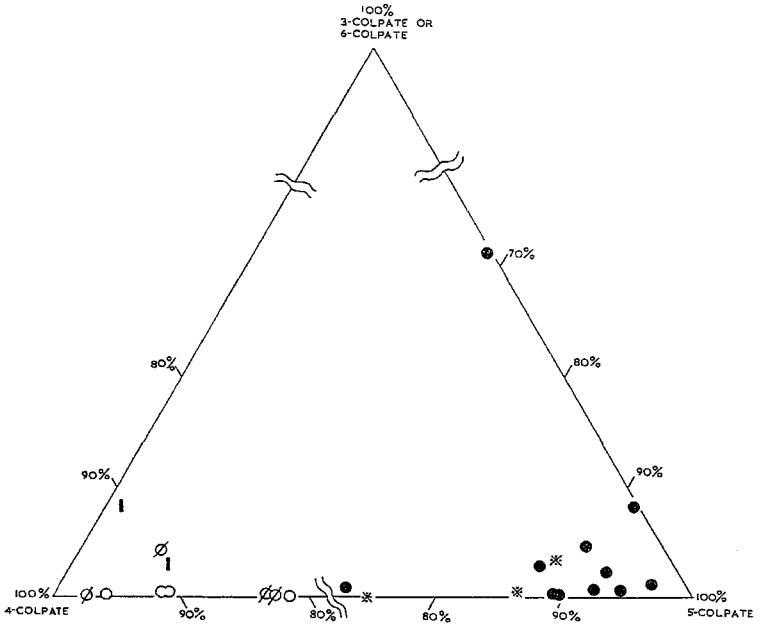

Fig. 4. Scatter diagram of mean percentage frequencies of grain types for populations listed in Table 2 I = *V lutea*; Ø = *V. curtisii*; ○ = *V. tricolor*; ● = *V. arvensis*; * = *V. nana*.

The scatter-diagram of mean frequencies of pollen types for each population (Fig. 4) shows the distinctness of these two groups. Within the left-hand group the assemblages of *V. curtisii* and *V. tricolor* are very similar and not readily distinguishable; *V. lutea* may differ from both by having a slightly higher frequency of 3-colpate grains with a corresponding reduction of 5-colpate grains but the data are too few to be certain. Within the right-hand group *V. arvensis* and *V. nana* are basically similar although *V. nana* may have a tendency towards a lower frequency of 5-colpate grains than *V. arvensis*, but the latter is rather widely-ranging in this respect.

Although within any one population the assemblages were relatively uniform in composition, differences from plant to plant did occur, but, with the exception of those already mentioned, these were not sufficient to cut across the limits of the groups defined above. It may be supposed that this variation was controlled partly by genetic differences and partly by environmental differences between individual plants. That this was not the whole explanation was shown by some chance counts on a series of duplicate slides. These are given in Table 3 and represent counts of slides made from different flowers of the same plant collected at the same time. The differences between duplicates in the ratio of 'most frequent grain type' to 'rest of assemblage' were mostly significant when tested statistically. This means that the differences were unlikely to have been the outcome of random sampling and suggests, perhaps, the conditions experienced by flowers on different branches of the same plant were sometimes sufficient to cause the small anomalies. It is, of course, unlikely that

TABLE 3. Variation in pollen assemblages of duplicate slides

Plant Number	*Date*	*Slide*	*Total grains scored*	*Percentage frequency of pollen grain types*				*Significance level*
				3-colpate	*4-colpate*	*5-colpate*	*6-colpate*	
T10/T4	Sept. 1956	a	763	0·13	89·38	10·49	—	Significantly different P <0·05> 0·01
		b	742	0·14	92·72	7·14	—	
T14/2	Oct. 1956	a	193	—	70·98	29·02	—	Not significantly different
		b	374	—	70·32	29·68	—	
A12/2	Sept. 1956	a	929	—	10·44	89·45	0·11	Not significantly different
		b	372	—	13·44	86·56	—	
A12/12	Sept. 1956	a	451	—	16·19	83·81	—	Not significantly different
		b	329	—	12·77	86·93	0·30	
A13/4	Sept. 1956	a	875	—	15·43	84·59	—	Significantly different P <0·001
		b	389	—	31·36	68·64	—	
A13/8	Sept. 1956	a	425	—	10·82	88·47	0·71	Significantly different P <0·01> 0·001
		b	621	—	5·47	93·08	1·45	
A29	Oct. 1956	a	624	—	0·96	95·51	3·53	Significantly different P <0·01>0·001
		b	293	—	—	99·66	0·34	
A33	Sept. 1957	a	589	—	15·28	84·72	—	Significantly different P <0·001
		b	410	—	7·23	92·77	—	

differences between flowers such as these would ever cause the assemblage of *V. arvensis* to resemble that of *V. tricolor*, or vice versa.

Data on the incidence of aborted grains and micrograins are also summarized in Table 2. Aborted grains were easily distinguishable from normal ones. They were smaller, contained little or no cytoplasm and in consequence lacked the granular appearance of the normal grains; the furrow configuration was present but usually inconspicuous because of their shrunken state; with methylene blue and eosin they usually stained a blue-green in contrast to the purple colour of the normal grains. Micrograins were somewhat similar to the aborted grains but different in that they were much smaller, lacked furrows, had very thin walls and consequently stained very lightly.

Whereas the aborted grains were merely pollen grains which had failed to develop completely and mature, the micrograins frequently owed their origin to the aggregation of cytoplasm around lagging and disorientated chromosomes or chromosome fragments. Since, then, micrograins are not strictly pollen grains they were not included in the total grain count for the calculation of percentage pollen abortion. Their incidence was expressed, however, as a percentage of the total grain count to indicate very roughly the incidence of chromosomal abnormalities during pollen formation.

A perusal of the data given in Table 2 will show that there was a tendency for the mean frequency of pollen abortion to be higher in the predominantly outbreeding taxa, *V. lutea*, *V. curtisii* and *V. tricolor*, than in the predominantly inbreeding taxa, *V. arvensis* and *V. nana*. This is probably a reflection of the greater heterozygosity for chromosomal interchanges in the former. (The anomalous high values of N45 were probably caused by the very heavy aphid infestation of the plants prior to pollen collection.) In contrast, the mean incidence of micrograins is more or less uniformly low throughout the range of populations where information is available.

4. Discussion

A comparison of the chromosome counts with the type of pollen assemblage for the different taxa shows a certain agreement between the two sets of data.

It should be noted that the two very variable and often confused species, *V. tricolor* and *V. arvensis*, are readily separable on the basis of both these characters. In fact, the close association between the two characters means that a determination of the pollen assemblage would be sufficient to place any doubtful specimen into one of the two species. Details of this character are now to be found in the second edition of Clapham, Tutin & Warburg's *Flora* (Warburg 1962). This should be of particular use with herbarium specimens where identification is often difficult because of poor preservation or undue fading of the flowers, and where counting of chromosomes cannot be contemplated.

In the case of the closely related taxa, *V. tricolor* and *V. curtisii*, neither the chromosome numbers nor the pollen assemblages offer grounds for their separation into two distinct species. This is contrary to the statement of Mullenders & Mullenders (1957) who maintained they were separate species largely on the basis of pollen assemblages. In the light of this present work it seems that, although the Mullenders emphasized their plants to be *V. tricolor* subsp. *tricolor*, they were in fact dealing with *V. arvensis* or something closely related to it. This is supported by the ecological details given by them which indicate that most of the plants were growing in calcareous soils. In Britain and Scandinavia at least, *V. tricolor* subsp. *tricolor* is confined to soils ranging from acid to neutral, whereas *V. arvensis* is characteristically found on calcareous soils (cf. Ferdinandsen 1918, Clausen 1922). Study of the AT-intermediates to be discussed in detail in another paper also indicates how this confusion probably arose.

The figures given in Erdtman (1952) showing that *V. arvensis* has 4-colpate with a few 5-colpate grains must also be based on a similar confusion over specific limits since this type of assemblage is found in *V. tricolor* and not *V. arvensis*. There is little reason for believing that Scandinavian plants of the latter species are any different from British examples in this respect, especially since Wittrock's original diagnoses of pansies using this character

refer in particular to Scandinavian plants, and they are in complete accordance with the present findings.

Finally, in addition to recommending the use of pollen assemblages for decisions on critical plants of *V. tricolor* and *V. arvensis*, the uncertain value of a high level of pollen abortion as a criterion for putative hybridity should also be emphasized. Clausen (1931) has already shown most interspecific hybrids to be relatively fertile, and very few to be completely sterile, whilst the data given above show that a certain amount of pollen abortion could be expected in at least some examples of the outbreeders, *V. tricolor*, *V. curtisii* and *V. lutea*, without the complications of interspecific hybridization.

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