STUDIES ON BRITISH PANSIES

II. THE STATUS OF SOME INTERMEDIATES BETWEEN *VIOLA TRICOLOR* L. AND *V. ARVENSIS* MURR.

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ABSTRACT

Plants morphologically intermediate between *Viola tricolor* L. and *V. arvensis* Murr. have, in the past, been interpreted either as hybrids between the two species or as 'stabilized' hybrid segregates. Several recent gatherings of such intermediates (AT-intermediates) and putative hybrids have been studied and compared with experimentally produced F_1 and F_2 hybrids.

Observations on the morphology and cytology of the experimentally produced hybrids largely confirm previous accounts. Pollen assemblages, not previously examined in any detail, were shown to be variable in both generations, ranging in character from that of *V. tricolor* to that of V. *arvensis*. Percentage pollen abortion was generally below 40% with a mean value of 22·8% for the F_1 and 24·4% for F_2. The incidence of micro-grains was usually greater than 2% with the mean value dropping from 7·4% in the F_1 to 3·9% in the F_2.

One gathering of intermediates from a mixed population of *V. tricolor* and *V. arvensis* was shown to be indistinguishable from experimentally produced hybrids. AT-intermediates from populations composed otherwise of *V. arvensis* or entirely of the intermediate form were shown to be indistinguishable from *V. arvensis* on the basis of chromosome number and pollen morphology.

Observations on successive generations of AT-intermediates grown in an experimental garden showed the original gatherings were composed of two general elements, (i) plants losing their intermediate features and becoming indistinguishable from the small-flowered *V. arvensis*, and, (ii) plants retaining their characteristics which were presumably genetically controlled.

The first group simulate AT-intermediates through phenotypic plasticity. It is suggested that the second group represent either the original outbreeding forms of *V. arvensis* or intermediate stages in the development of the inbreeding régime typical of *V. arvensis*, rather than 'stabilized' hybrid segregates. In consequence of this it seems advisable to widen the circumscription of *V. arvensis* to include these extreme morphological forms.

1. INTRODUCTION

During a study of the cytology and pollen morphology of some British pansies (Pettet 1964) it was found convenient to divide the *Viola tricolor–V. arvensis* complex of annual pansies into three main groups, viz. (i) *V. tricolor*, (ii) *V. arvensis*, and (iii) intermediates between these, designated 'AT-intermediates'. The last-mentioned had cream, or predominantly cream flowers, the upper petals longer than the upper sepals (petals usually $1\frac{1}{4}$–$1\frac{1}{2}$ × the length of the sepals) and stylar flap intermediate in development between those of *V. tricolor* and *V. arvensis*. In brief, the AT-intermediates had flowers resembling those of *V. tricolor*, except that they were cream-coloured, had a smaller stylar flap and a somewhat reduced ratio of upper petal to upper sepal. As was previously stated, the AT-intermediates so defined should not be considered a distinct entity—only one of convenience encompassing those plants not readily ascribed to either *V. tricolor* or *V. arvensis*. Some individuals can be equated with the cream-flowered species or varieties of Drabble's *Tricolores* (Drabble 1909, 1927a), e.g. *V. contempta* Jord., *V. variata* Jord. var. *sulphurea* Drabble, but others are not so readily identified with these microspecies. Wittrock (1897), Kristofferson (1923) and Clausen (1922, 1926) have reported similar plants from a number of different places on the Continent and a study of specimens in the herbaria of Kew and

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the British Museum (Nat. Hist.) has shown that AT-intermediates occur not infrequently in Britain.

Clausen (1922, 1926) examined a number of intermediates occurring in mixed populations of *V. tricolor* and *V. arvensis* and showed them to be hybrids on cytological evidence and on comparison with experimentally produced hybrids. Others occurring without either parent were found to be cytologically regular and these Clausen suggested were 'stabilized' hybrid segregates of long standing. By way of confirmation for this conclusion Clausen showed how, after a few generations, some segregates of intermediate morphology derived from the interspecific cross *V. tricolor* × *V. arvensis* were morphologically and cytologically relatively constant and possessed chromosome numbers either similar to the *V. tricolor* parent or higher than the *V. arvensis* parent (Clausen, 1924). The report of aberrant chromosome numbers for two cream-flowered *Tricolores* pansies by Fothergill (1944), viz. *V. variata* var. *sulphurea* with $2n = 26$ and $2n = 28$, *V. contempta* with $2n = 40$, might suggest that stabilized hybrid segregates as visualized by Clausen do actually occur in Britain. It should be noted, however, that morphologically similar plants with large cream flowers have been derived from an intraspecific cross between two lines of *V. arvensis* (Clausen 1931) and these had a lower chromosome number ($2n = 32$) than the parent lines with $2n = 34$ as a result of the loss of a pair of chromosomes during meiosis of the initial hybrid.

Evidence for Clausen's conclusion that AT-intermediates represent segregates from past hybridizations is thus strong and this view is reflected in certain of the more recent treatments of the complex, e.g. some of the cream-flowered members of Drabble's group, the *Tricolores*, are quoted as synonyms for the interspecific cross *V. tricolor* × *V. arvensis* in the *List of British Vascular Plants* (Dandy 1958).

During work on British pansies a number of gatherings of putative hybrids and AT-intermediates was made and a critical comparison of these with a number of experimentally produced hybrids between *V. tricolor* and *V. arvensis* carried out. In view of the light that this comparison has thrown on the nature of the variation in the *V. tricolor–V. arvensis* complex and the reported importance of interspecific hybridization to this problem, the results of this aspect of the work form the body of this paper. Clausen has already described experimentally produced hybrids but, to facilitate appropriate comparisons, a summary of the main features of the experimental hybrids used in this study has also been included. Those points not pursued in any detail by Clausen have been elaborated but for all features of the experimentally produced hybrids fuller details may be found in Pettet (1960).

2. Experimentally produced hybrids

Cross-pollination of flowers of *V. arvensis* and *V. tricolor* was easily effected with a sterilized needle about 4–5 days after emasculation of the flowers, and plenty of viable seed normally obtained. In hot and dry weather the emasculated flowers were slipped into a piece of cellophane drinking-straw, sealed at one end to avoid losses from the drying-out of the flowers. When the fertilized capsules started swelling the straws were replaced by small muslin bags to collect the seed and prevent the violent discharge of the capsule.

The F_2 generation of the cross was obtained simply by selfing the F_1 hybrids.

(a) *Morphological features*

Of those features used to separate *V. tricolor* from *V. arvensis*, floral characters appeared to be most useful in distinguishing F_1 and F_2 hybrids from the parental species and these are summarized in Table 1. Leaf and stipule characters were generally of little value because of their variation in both parent species, especially in *V. arvensis*. Occasionally stipule characters were of value in distinguishing F_1 hybrids from the two parental species. Where the *tricolor* parent had a narrow, non-leafy stipule mid-lobe and the *arvensis* parent had a wide, leafy stipule mid-lobe, it was possible to recognize the intermediate type of stipule

mid-lobe in the hybrid. Usually, however, the stipule characters were rarely as clear cut as this and thus of limited value.

The floral characters of the F_1 have been found useful in distinguishing the hybrid from the parental species in the field. The slow change in flower colour some time after the flowers have opened—1–2 days or more if the weather is hot—is particularly striking once recognized. There is, of course, a similar colour change in the flowers of *V. tricolor* but it is much faster and the changes occur as the flower unrolls.

TABLE 1. Floral characters of the F_1 and F_2 generations of the cross *V. tricolor* × *V. arvensis*.

Character	*F_1 generation*	*F_2 generation*
Vertical length of flower	13–20 mm, mostly *c.* 15 mm	(5–) 7–8–9 (–13) mm
Flower colour:		
(i) Young flowers	All petals cream	All petals white, or whitish suffused with very pale blue, or occasionally pale cream
(ii) Old flowers	Upper petals blue but lacking deep blue or blue-purple typical of *V. tricolor*; lateral petals cream, suffused with pale blue, or occasionally all cream; lower petals all cream, or cream edged with pale blue	Usually all petals bluish, sometimes deep blue; or upper petals pale blue, lateral petals whitish or very pale cream, lower petals very pale cream
Length of upper petal: Length of upper sepal	(1–) $1\frac{1}{4}$–$1\frac{1}{2}$ (–1): $1\frac{3}{4}$	($\frac{1}{2}$–) $\frac{3}{4}$–$1\frac{1}{2}$: 1
Length of spur: Length of sepaline appendages	(1–) $1\frac{1}{4}$–$1\frac{1}{2}$: 1	$1\frac{1}{4}$–$1\frac{3}{4}$ (–$2\frac{1}{2}$): 1
Development of stylar flap	Intermediate between those of parent species	Variable, mostly intermediate but some almost as large as that of *V. tricolor* and other reduced

Because of the variability to be seen in the F_2 generation it might not be possible to identify every example as belonging to this generation on purely morphological features unless the parents were accompanied by both filial generations. The general impression was that of small, pale, *tricolor*-like flowers on rather poorly developed plants. Amongst the families grown, however, one feature was noticed which may occur generally throughout the generation and thus be an aid in distinguishing the F_2 in the field. In contrast with the F_1 and parental plants where the upper petals were ovate-oblong and were held more or less vertically and overlapped to varying degrees, the flowers of the F_2 had the upper petals narrowly oblong and widely aligned at angles up to approximately 45° from the vertical. This produced a different aspect to the flower and one which should be fairly easy to observe in the field.

(b) *Pollen assemblages*

The method of preparation and examination of pollen has previously been described in detail (Pettet 1964).

In contrast with the parental species the pollen assemblages of both filial generations of hybrids showed a great variability in the development of the grains. Although the number of colpae was normally obvious, it was possible to find a gradation from the relatively 'perfect' grains to the fully aborted ones, with grains in the intermediate stages showing various degrees of deeper staining and lesser expansion. This gradation in development was also reflected in the marked variability of grain size. In addition, it was occasionally possible to find grains clearly abnormal in their development, e.g. grains fused together by a common part of the exine or grains with large numbers of poorly developed colpae.

TABLE 2. Pollen assemblages and incidences of pollen abortion and micro-grains of F_1 Hybrids

Parents of Cross ♀ ♂	*Plant no.*	*Slide no.*	*Number of grains scored*	*Percentage frequency of pollen-grain types*				*Number of grains counted*	*Percentage of aborted pollen*	*Percentage of micro-grains*
				3-*colpate*	4-*colpate*	5-*colpate*	6-*colpate*			
T10 × A10	1	i	196	—	18·88	80·10	1·02	577	64·65	12·99
		ii	321	—	7·16	81·93	10·90	553	37·98	7·96
		iii	264	—	6·06	84·09	9·85	418	33·01	2·87
		iv	290	—	15·17	81·72	3·10	418	27·99	3·11
		v	295	—	4·41	91·53	4·07	440	31·36	3·86
T10 × A10	2	i	304	—	67·43	32·57	—	338	10·06	2·66
		ii	844	—	58·06	41·94	—	943	10·07	2·23
		iii	842	—	69·72	30·28	—	940	9·47	3·09
		iv	432	—	49·31	50·69	—	504	13·09	1·59
		v	691	—	76·84	23·16	—	771	7·52	2·08
		vi	479	—	66·81	33·19	—	591	16·24	2·03
		vii	602	—	55·98	44·02	—	692	11·85	2·46
T10 × A10	3	i	191	—	41·36	58·64	—	393	50·64	4·83
T10 × A10	4	i	430	—	50·70	49·07	0·23	568	23·06	4·05
T10 × A10	5	i	352	—	17·61	80·49	1·42	485	26·80	4·12
T10 × A12	1	i	912	—	87·06	12·94	—	1,132	17·67	8·92
		ii	647	—	88·25	11·75	—	761	14·45	8·41
A33 × T10	1	i	553	0·36	89·15	10·49	—	593	30·01	14·33
A33 × T10	2	i	433	1·62	96·99	1·39	—	487	10·06	13·76
A36 × T10	1	i	632	—	59·18	40·66	0·16	699	8·58	9·73
A42 × T10	1	i	653	—	74·96	25·04	—	660	13·03	6·36

(i) *F_1 generation*

The pollen assemblages and incidences of pollen abortion and micro-grains of the various F_1 hybrids are given in Table 2. These show an unexpected and wide variability in contrast to the relative uniformity of the gross morphological features of the F_1 hybrids. Some plants have assemblages indistinguishable from those of *V. tricolor* (cf. Pettet 1964), e.g. crosses involving A12, A33 and A42; others have assemblages resembling those of *V. arvensis*, e.g. crosses involving A10 (Nos. 1 and 5); and yet a few have 4-colpate and 5-colpate grains in approximately similar proportions as Clausen (1922) supposed hybrids o have, e.g. crosses involving A36 and A10 (Nos. 2, 3 and 4).

TABLE 3. Direction of cross and type of pollen assemblage.

Pollen-contributing parent	*Predominantly* 'tricolor' *assemblage*	*Intermediate assemblage*	*Predominantly* 'arvensis' *assemblage*
tricolor	3	1	–
arvensis	1	3	2

It can be seen from Table 3 that the direction of the cross seems to control the pollen assemblages of the progeny. Where the pollen-contributing parent was *V. tricolor* the assemblages of the F_1 tend to resemble a *tricolor* assemblage; similarly, when *V. arvensis* was the pollen-contributing parent, there was a tendency, although less marked, for the F_1 assemblage to resemble an *arvensis* assemblage. Unfortunately the number of plants involved was too small to allow a more definite conclusion.

The percentage abortion was generally low. Except for two high values, viz. 64·65% and 50·64%, all the values were below 40% with the majority below 30%. These values are similar to those already recorded for certain populations of *V. tricolor*. The incidence of micro-grains, on the other hand, was higher than found for either parent species, where it was usually less than 1%.

(ii) *F_2 generation*

The pollen assemblages and incidences of pollen abortion and micro-grains of the two experimentally produced families of the F_2 generation are given in Table 4. In many respects these assemblages were similar to those of the F_1 hybrids, ranging in character from those resembling *V. tricolor* to those resembling *V. arvensis*, but they differed from the F_1 in the tendency for the majority of the assemblages to cluster about the intermediate state. From Tables 4 and 5 it may be seen that the percentage abortion in the F_2 was similar to that in F_1, but that the incidence of micro-grains in the F_2 was decidedly lower.

As a character for distinguishing hybrids from their parent species pollen assemblages need to be used with caution; not all assemblages will be intermediate in character and some will resemble *V. tricolor* or *V. arvensis* to a greater or lesser extent. However, a combination of a moderate degree of pollen abortion (frequency greater than 10%) and an unusually high incidence of micro-grains (frequency greater than 1% of the total grain assemblage) would seem sufficient to identify at least the majority of F_1 and F_2 hybrids whose assemblages of functional pollen resembled one or other of the parent species. Irregular size and development of the grains, with the presence of the occasional abnormal grain, would provide further confirmation of hybridity, although the former features may not be easy to determine in pollen from old herbarium material where expansion and staining of the grains is not always uniform.

TABLE 4. Pollen assemblages and incidences of pollen abortion and micro-grains of F_2 hybrids

Parent hybrid	*Plant no.*	*Slide no.*	*Number of grains scored*	*Percentage frequency of pollen-grain types*				*Number of grains counted*	*Percentage of aborted grains*	*Percentage of micro-grains*
				3-*colpate*	4-*colpate*	5-*colpate*	6-*colpate*			
T10×A10/1	1	i	150	—	59·33	40·00	0·67	334	43·11	7·49
		ii	114	—	70·18	29·82	—	223	26·91	3·14
		iii	133	—	50·38	49·62	—	338	39·94	4·73
		iv	235	—	60·00	39·57	0·43	412	31·55	3·16
	2	i	92	—	34·78	65·22	—	336	54·46	0·60
		ii	78	—	57·69	42·31	—	351	56·69	2·56
	3	i	271	—	82·29	17·71	—	271	8·63	4·06
		ii	266	—	77·44	22·56	—	266	8·39	3·76
	4	i	85	—	10·59	89·41	—	301	68·44	6·98
	5	i	216	0·93	50·00	48·61	0·46	282	12·06	3·90
	6	i	258	—	43·02	56·59	0·39	407	26·04	8·60
	7	i	249	—	57·43	42·57	—	290	16·38	1·38
	8	i	239	—	61·92	38·07	—	323	20·74	0·93
	9	i	457	—	39·17	60·61	0·22	714	18·77	0·98
	10	i	241	—	17·84	81·33	0·83	444	14·98	4·25
	11	i	376	—	41·75	57·98	0·27	669	15·25	2·54
T10×A10/2	1	i	529	—	33·46	66·54	—	640	14·84	3·59
	2	i	491	—	49·69	50·31	—	558	10·57	1·08
	3	i	661	—	45·54	54·46	—	747	10·84	0·80
	4	i	93	—	4·30	73·12	22·58	332	42·77	14·76
	5	i	454	—	39·21	60·57	0·22	600	19·50	2·50

(c) *Cytology*

The fixation of young flower buds and the staining of the pollen mother cells (PMC) have been described in a previous paper (Pettet 1964).

Chromosomes in the PMCs of the hybrids were sometimes difficult to stain satisfactorily so that not all the available material could be studied in detail. Sufficient buds stained well enough, however, to give a representative sample of the available experimental hybrids.

TABLE 5. Changes in the mean incidence of pollen abortion and micro-grains in F_1 and F_2.

	Mean Values	
	F_1 (N = 10)	F_2 (N = 16)
Percentage pollen abortion	22·84	24·42
Percentage micro-grains	7·43	3·90

(i) *Meiosis in the F_1 generation*

Although chromosome pairing at first metaphase of meiosis was variable no multivalents were observed in the F_1 hybrids. Most frequently 13 or 14 bivalents were formed with 4 or 2 univalents left unpaired respectively; less frequently 12 bivalents associated with six univalents were observed when pairing was less complete, or 15 bivalents and no univalents when pairing was complete.

Subsequent behaviour of the bivalents during anaphase and telophase was usually normal with regular segregation to the poles. At times, however, one to several bivalents could be seen lagging in the equatorial region (Fig. 1) and, since disorientated bivalents were often found in the peripheral cytoplasm during the later phase of meiosis, it would

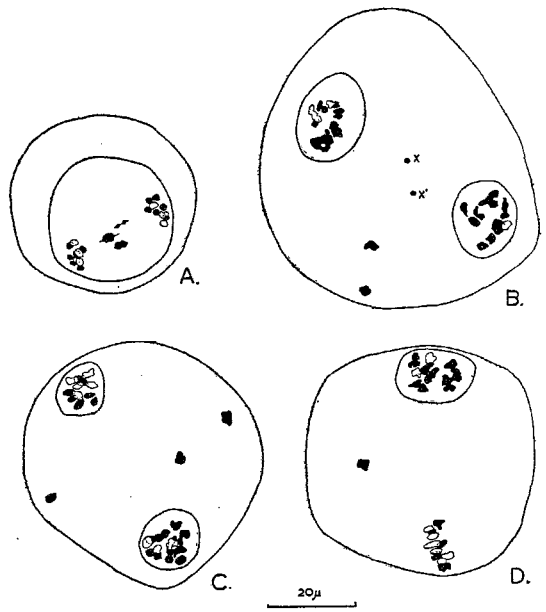

Fig. 1. PMC meiosis in F_1 generation of *V. tricolor* × *arvensis*. (a) A30 × T10. Late stage of first anaphase with two lagging bivalents and a dividing univalent. (b) A33 × T10. Interkinesis with two bivalents and a divided univalent (X and X^1) which lagged during first division of meiosis. (c) A33 × T10. Interkinesis with three bivalents lying free in the cytoplasm. (d) A33 × T10. Nonsynchronization of divisions within the same PMC. Interkinesis and early second metaphase with a bivalent lying free in the cytoplasm from the first division of meiosis.

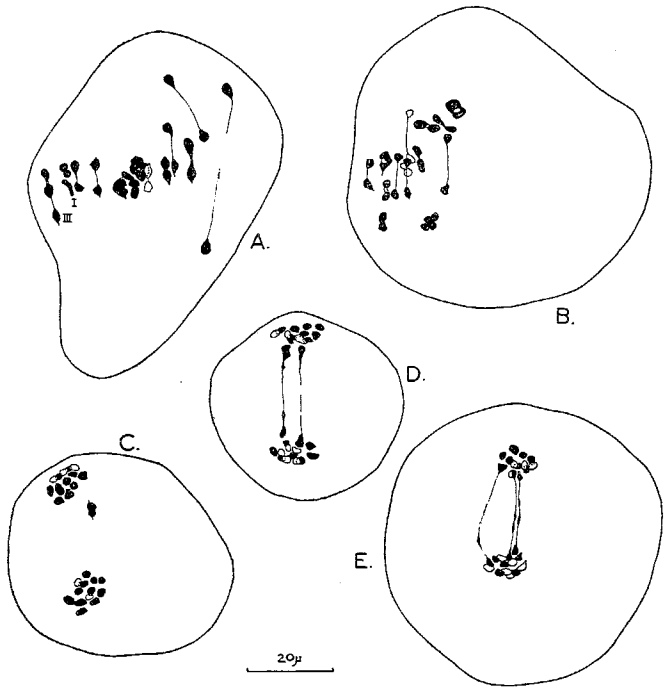

Fig. 2. PMC meiosis in F_2 generation of *V. tricolor* × *arvensis*. (a) $2n = 28$. First metaphase. Twelve bivalents, one trivalent and one univalent. (b) $2n = 28$. First metaphase. Complete pairing with the formation of 14 bivalents. (c) $2n = 28$. Late stages of first anaphase. One lagging bivalent. (d) $2n = 27$. Late stage of first anaphase. Two chromatid bridges with 13 chromosomes at upper pole and 14 at lower pole. (e) $2n = 28$. Late stage of first anaphase. Three chromatid bridges.

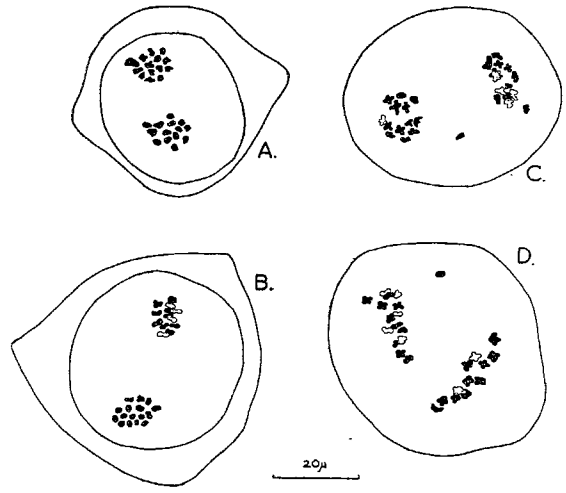

Fig. 3. PMC meiosis in F_2 generation of *V. tricolor* × *arvensis*. (a) $2n = 28$. Second metaphase. Equal segregation of chromosomes in first division with 14 chromosomes going to each pole. (b) $2n = 29$. Second metaphase. Unequal segregation of chromosomes during first division with 13 in the upper complement and 16 in the lower complement. (c) $2n = 28$. Second prometaphase. Univalent from first division lying disorientated in cytoplasm. Fourteen chromosomes in right-hand complement, 13 in left-hand complement. (d) $2n = 28$. Beginning of second metaphase. Unequal segregation of chromosomes during first division with 15 in left-hand complement and 12 in right-hand complement. Disorientated univalent from first division lying in cytoplasm.

appear that these lagging bivalents often failed to separate completely and were lost to the following phases of meiosis.

The behaviour of the unpaired univalents varied, Most frequently they remained disorientated in the cytoplasm and were to be observed as somewhat ill-defined chromosomes lying apart from the general cluster of chromosomes. Occasionally unpaired univalents became orientated on the metaphase plate and moved to one or other of the poles, subsequently to participate in the second division of meiosis. Very rarely univalents reorientated on the metaphase plate and divided precociously to give two very small chromosomes which, in the few cells in which they were observed, may have moved to the poles to become included in the subsequent divisions of meiosis. The situation shown in Fig. 1 suggests, however, that the division of the univalents sometimes occurred too late for the products to reach the poles before telophase and interkinesis.

Exclusion of disorientated and lagging univalents and bivalents from the main chromosome mass during meiosis would, of course, lead to a reduction in the chromosome numbers of the developing tetrads. Provided this produced no markedly deleterious effect on their development this would lead to the formation of pollen grains with chromosome numbers lower than the haploid number of the parent, and eventually with numbers resembling that of *V. tricolor*. Very occasionally irregular segregation of chromosomes during anaphase produced complements containing numbers higher than the haploid number of the parental hybrid and, no doubt, these would give rise to pollen grains with increased chromosome numbers. The infrequency of such events, however, makes it unlikely that they materially affected the general trend towards a decrease in number and possibly a stabilization at $n = 13$ as observed by Clausen (1922, 1926).

(ii) *Meiosis in the F_2 generation*

The loss of chromosomes during meiosis in the F_1 hybrids was reflected in the chromosome numbers observed in the F_2 generation. Most plants had $2n = 28$, but plants with $2n = 27$ and $2n = 29$ were occasionally found (Figs. 2 and 3).

In contrast with the F_1 hybrid, chromosome pairing during meiosis (plants with $2n = 28$) was most frequently regular and complete. Multivalents were not observed except for a single example of a trivalent observed in one PMC of a plant with $2n = 28$ (Fig. 2). This regularity of chromosome pairing suggests that chromosome loss and segregation in both PMCs and megaspore mother cells (MMCs) of the F_1 had not been completely haphazard and that homologous chromosomes were rarely included in the same complement.

At times, however, chromosome pairing and segregation were not completely regular. As in the F_1, two or more chromosomes sometimes failed to pair, or bivalents remained disorientated during first metaphase and later passed into the peripheral cytoplasm. These malfunctioning chromosomes, and possibly the extra univalents observed in the odd numbered plants, might well have represented the extra *arvensis* chromosomes which paired autosyndetically and segregated regularly in the F_1.

Even though chromosome segregation proceeded with greater regularity in the F_2, counts made at second metaphase showed that unequal chromosome segregation still occurred from time to time.

One noticeable difference between meiosis in the F_1 and F_2 deserves mention. During first and second anaphases in the F_1 chromatid bridges were rare, whereas in the F_2 they were more frequent and sometimes even numerous. The reasons for this difference are not immediately obvious although several possibilities exist. Perhaps the most likely explanation is that both F_1 and F_2 were heterozygous for a similar number of chromosomal interchanges but more frequent chiasmata formation in the F_2 made them more readily detectable in that generation.

3. Putative hybrids and AT-intermediates

Details of the location and numbers of putative hybrids and AT-intermediates which were available for study are given in Table 6. Most were found singly or in relatively small

numbers in populations otherwise composed solely of plants typical of *V. arvensis*, although two collections came from populations composed entirely of AT-intermediates and one from a mixed population of *V. tricolor* and *V. arvensis*. Mixed populations have been recorded from time to time in the past but only the one example (Table 6) was found in the 3 years' fieldwork (1956–59) of the present study. The presence of these AT-intermediates in such a population suggested hybridization had taken place under field conditions.

TABLE 6. Putative hybrids and AT-intermediates available for study.

Plant No.	*Locality*	*Habitat*	*Frequency*
T10/A10	Nr. Aberystwyth, Cards.	Derelict field	Slightly under 1% of population composed of both *V. tricolor* and *V. arvensis*
A2/24	Southampton, Hants	Disturbed gravel	Single plant with 23 others which were typical of *V. arvensis*
A5/6	Southampton, Hants	Corporation Rubbish Tip	Single plant with 8 others which were typical of *V. arvensis*
A12/1	Durham, Co. Durham	Allotments	Single plant with 19 others which were typical of *V. arvensis*
A30	Ruislip. Middlesex	Barley field	Whole population of this form
A36/11, 12	Nr. Hursley, S. Hants	Barley field on chalk	Two plants in a very large population of plants typical of *V. arvensis*
A37/10	Hursley, S. Hants	Barley field on chalk	Single plants in very large population of plants typical of *V. arvensis*
A38i	Rockland, Norfolk	Barley field	Single plant in very large population of plants typical of *V. arvensis*
A41	Black Isle, Cromarty, Scotland	Root-crop field	Whole population of this form

(a) *Putative hybrids in mixed population of* V. tricolor *and* V. arvensis

A number of the putative hybrids were removed from the population to the garden at Southampton but only three plants survived the transfer. These were morphologically indistinguishable from F_1 hybrids subsequently produced from the two supposed parent species of the same population. All three plants were found to have a chromosome number of $2n = 30$ and a meiotic behaviour closely resembling the F_1 hybrids already described. The pollen assemblages of two plants were intermediate in character between those of *V. tricolor* and *V. arvensis*, while the third plant had an assemblage which more closely resembled that of *V. tricolor*. There is little doubt, then, that these three plants were F_1 hybrids. Since all putative hybrids in this population were relatively uniform there is no evidence to suggest they represented anything other than F_1 hybrids. This was confirmed by a critical examination of the herbarium specimens taken when the population was first studied which failed to reveal any F_2 or backcross hybrids.

(b) *AT-intermediates from unmixed populations*

The more important gross morphological features of the AT-intermediates from unmixed populations are given in Table 7. Although not completely identical with F_1 or F_2 hybrids (Table 1), they resembled them closely enough to raise doubts about their identity. To investigate the possibility that they were hybrids or hybrid-derivatives these plants were removed from the field for further observations and experimental work.

(i) *Observations on phenotypic plasticity and genetic heterozygosity*

A number of observations on phenotypic plasticity and genetic heterozygosity were made whilst growing these plants for experimental purposes and these are summarized in

TABLE 7. Morphological characteristics of AT-intermediates

Plant no.	*Stipule shape*	*Shape of stipule mid-lobe*	*Flower colour*	*Vertical length (mm)*	*Upper petal/ Upper sepal*	*Spur/sepaline appendage*	*Stylar flap*
A2/24	Pinnate	Lanceolate, leafy and crenately notched	All petals cream	14	1·25–1·5:1	1–25:1	Present but reduced
A5/6	Pinnate	Ovate-lanceolate, leafy and crenately notched	All petals cream	15	1·5:1	1–1·25:1	Present but reduced
A12/1	Palmate-pinnate	Ovate-lanceolate, leafy and crenately notched	All petals cream	17–18	1·75–2:1	1·25–1·5:1	Present, intermediate in size between that of *V. tricolor* and *V. arvensis*
AT30	Pinnate	Lanceolate, leafy and crenately notched	Upper petals pale blue; lateral and lower petals cream tinged blue	16–18	1·5–1·75:1	1:1	Intermediate
A36/11, 12	Pinnate	Ovate-lanceolate, leafy and crenately notched	All petals cream	15	1·25–1·5:1	1:1	Present but reduced
A37/10	Pinnate	Ovate-lanceolate, leafy and crenately notched	All petals cream	15	1·25–1·5:1	1·25:1	Present but reduced
A38i	Pinnate	Ovate-lanceolate, leafy and crenately notched	Upper petals deep purple; lateral and lower petals cream	18	1·5–1·75:1	1·5–2:1	Present but very poorly developed
AT41	Palmate-pinnate	Lanceolate, hardly leafy, entire	All petals cream	17–18	1·5–1·75:1	1·25–1·5:1	Present, intermediate in size between that of *V. tricolor* and *V. arvensis*

TABLE 8. Summary of observations on phenotypic plasticity and genetic heterozygosity of AT-intermediates (less than 40 plants/generation are indicated in brackets)

Plant no.	*Morphological changes after removal to experimental garden*	*Morphological features of F_1 generation*	*Morphological features of F_2 generation*	*Morphological features of F_3 generation*
A2/24	Plant did not survive long enough for any changes to be observed	Flowers smaller; resembling those of other members of original population; uniform with no apparent segregation (10 plants)	Flowers as in F_1; ± typical of original population; uniform	Flowers as in F_1 and F_2; uniform
A5/6	Flowers initially large but becoming reduced in size by late autumn until resembling those of original population	Flowers small; resembling those of other members of original population; uniform with no apparent segregation	Flowers as in F_1; uniform	—
A12/1	Retained features throughout succeeding winter	Retained features of parent plant except flowers were slightly larger (1 plant)	Retained features of parent plant; uniform (36 plants)	Flowers as in F_1 and F_2; uniform
AT30	Retained features throughout succeeding winter	Retained features of parent population; uniform	Retained features of parent population; uniform	—
A36/11, 12	Plants did not survive long enough for any changes to be observed	Flowers smaller; indistinguishable from those of other members of original population; uniform with no apparent segregation	Flowers as in F_1; uniform	—
A37/10	Plant did not survive long enough for any changes to be observed	Retained features of parent plant; uniform	Flowers as in F_1; uniform	—
A38i	Plant did not survive long enough for any changes to be observed	Segregation for flower colour and size (see Table 9) (14 plants)	—	—
AT41	Retained features throughout succeeding winter	Retained features of parent population; uniform	Flowers as in F_1; uniform	—

Table 8. From this it is clear that the AT-intermediates do not form a single, coherent entity. They were, in fact, composed of two general elements:

A. Plants which lost the characteristics of AT-intermediates, becoming indistinguishable from the small-flowered *V. arvensis* with which they were originally growing. The original characters of these plants obviously formed extreme phenotypic expressions of those typical of *V. arvensis*.
(A2/24, A5/6, A36/11 and A36/12).

B. Plants which retained their characteristics ± unchanged through several generations. These presumably had a genetic basis. Compared with group A, plants of this group had flowers tending to have a somewhat larger upper petal/upper sepal ratio and a better developed stylar flap (cf. Table 7).
(A12/1, A30, A37/10 and A41).

Of those AT-intermediates examined only one, A38i, appears to have been heterozygous for flower size and colour, the features of the F_1 being summarized in Table 9.

TABLE 9. Segregation of floral characters in fourteen plants of F_1 of A38i

Number of plants	*Colour of upper petals*	*Upper petal/ Upper sepal*	*Spur/Sepal appendage*	*Honey-guide streaks*
4	Cream	1·5:1	1·5–2:1	unbranched
2	Cream	1·5:1	1·5–2:1	branched
3	Cream	1–1·25:1	1·5–2:1	unbranched
1	Cream	1·5:1	1·25:1	unbranched
1	Cream	1·5:1	1·25:1	branched
1	Cream	1:1	1·5–2:1	unbranched
1	Cream with large, deep-purple blotch covering *c.* ⅛ petal	1·5:1	1·5:1	branched
1	Cream with large, deep purple blotch covering *c.* ¼ petal	1·25:1	1·5:1	unbranched

(ii) *Pollen assemblages*

With the exceptions of A5/6 and A38i, the pollen assemblages of all plants taken from the field have been examined. In the case of A38i eight plants of the F_1 generation were examined instead.

It will be seen from the counts given in Table 10 that the AT-intermediates had assemblages closely resembling those of *V. arvensis* (cf. Pettet 1964). Penta-colpate grains were most abundant, comprising more than 80% of the total assemblage although in the F_1 of A38i the proportion was slightly lower, Tetra-colpate and 6-colpate grains occurred as subsidiary types, the former being the more frequent. The incidence of pollen abortion was also roughly at the same level as in *V. arvensis* rather than in the experimental hybrids, and the same applies to the incidence of micro-grains where these were counted.

Although no detailed counts for the succeeding generations of these plants were made, a few sample counts showed, with one exception, no striking changes in the relative proportions of grain types. In the case of A12/1 counts showed certain anomalous features and they are listed separately in Table 11.

The counts from the parent plant, A12/1, showed initially an assemblage similar to that of *V. tricolor*, but later one midway between those of *V. tricolor* and *V. arvensis*. Counts from flowers of the single F_1 plant generally confirmed the first count, indicating assemblages similar to those of *V. tricolor*, except for the first slide which had an assemblage midway between those of the two species. This was rather puzzling, and in order to eliminate any variation in the assemblages caused by seasonal differences, the entire sample from the F_2 was collected at the same time.

TABLE 10. Pollen assemblages and incidences of pollen abortion and micro-grains of AT-intermediates (excluding A12/1 and its progeny)

Population no.	*Plant no.*	*Number of grains scored*	*Percentage frequency of pollen-grain types*				*Number of grains counted*	*Percentage of aborted grains*	*Percentage of micro-grains*
			3-*colpate*	4-*colpate*	5-*colpate*	6-*colpate*			
A2	24	257	—	1·17	98·83	—	263	0·38	0·00
A30	1	94	—	11·70	88·30	—	143	6·10	Not scored
	2	407	—	5·41	94·59	—	521	3·26	Not scored
A36	11	342	—	5·85	93·57	0·58	396	1·26	Not scored
	12	362	—	3·31	96·69	—	381	0·52	Not scored
A37	10	192	—	14·06	85·94	—	221	6·33	Not scored
A41	1	117	—	8·55	91·45	—	277	0·36	Not scored
	2	96	—	11·46	88·54	—	194	0·52	Not scored
	3	285	—	13·68	86·32	—	419	0·00	Not scored
	4	169	—	10·06	89·94	—	376	0·00	Not scored
A38:	1	667	—	28·04	71·66	0·30	670	0·30	0·29
(F_1 generation)	2	485	—	26·39	73·40	0·21	492	1·22	0·20
	3	220	—	14·09	85·45	0·46	221	0·45	0·45
	4	576	—	2·43	94·44	3·13	597	0·50	0·84
	5	388	—	22·68	77·32	—	391	0·51	0·00
	6	629	—	10·49	89·35	0·16	632	0·16	0·00
	7	237	—	27·00	72·57	0·42	242	1·24	0·00
	8	484	—	29·13	70·25	0·62	512	5·47	3·13

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TABLE 11. Pollen assemblages and incidences of pollen abortion of micro-grains of A12/1 and its progeny

Generation	*Plant no.*	*Slide no.*	*Number of grains scored*	*Percentages frequency of pollen-grain type*				*Number of grains counted*	*Percentage of aborted grains*	*Percentage of micro-grains*
				3-*colpate*	4-*colpate*	5-*colpate*	6-*colpate*			
A12/1	1	i	712	—	80·06	19·94	—	744	3·76	0·40
		ii	751	—	49·40	50·60	—	817	7·47	0·00
F_1	1	i	1,025	—	57·95	42·05	—	1,045	1·34	0·00
		ii	1,395	—	74·45	25·45	—	1,418	0·99	0·14
		iii	1,213	—	72·88	27·12	—	1,221	0·66	0·16
		iv	1,051	—	80·49	19·51	—	1,066	1·41	0·19
F_2	1	i	665	—	43·76	56·24	—	759	12·12	0·00
	2	i	660	—	29·39	70·61	—	697	3·16	0·14
	3	i	530	—	23·02	76·98	—	579	6·39	0·52
	4	i	198	—	29·80	70·20	—	262	22·90	0·76
	5	i	351	—	22·79	77·21	—	376	6·38	0·27
	6	i	631	—	27·42	72·43	0·16	647	1·55	0·00
	7	i	556	—	26·44	73·56	—	566	1·24	0·00
	8	i	677	—	40·62	59·38	—	688	1·02	0·00
	9	i	574	—	30·31	69·69	—	589	1·36	0·34
	10	i	356	—	51·69	48·31	—	364	1·92	0·00

Counts from most plants of this generation showed assemblages similar to those of *V. arvensis* but plants 1, 8 and 10 had assemblages midway between the two main types. In view of the comparative uniformity in gross morphological features of the various generations of this line and the absence of any similar variation in counts from other plants, except perhaps in those of hybrids, this variation was unexpected. Unlike the hybrids, however, the variation here was not associated with cytological disturbances for, as shown below, chromosome behaviour at meiosis was regular.

It is thus difficult to explain these changes, especially as the genetical basis of pollen polymorphism is not fully understood. The variation in the assemblages recorded for the parent plant from the original population, and the comparative uniformity in the reversed assemblages of the F_2, suggest the differences here are not primarily controlled by genic segregation. Most of the variation must instead be explicable on the grounds of the plasticity of pollen morphology of this particular line of plants—a unique situation not observed elsewhere.

TABLE 12. Chromosome numbers of AT-intermediates

Population number	*Plant no.*	*Chromosome number*	
		n (*PMC*)	2n (*RT*)
A2	24	17	34
A5	6	17	—
A12	1	17	—
AT30	1–4	17	—
A36	11	17	34
	12	17	—
A37	10	17	—
A38i	1	17	—
AT41	1–5	17	—

(iii) *Cytology*

All plants collected from the field were examined cytologically and their chromosome numbers are recorded in Table 12. In every case the number is identical with that of *V. arvensis* and, like the latter, meiosis in all plants was regular with the formation of seventeen bivalents. Only very occasionally were PMCs found with a pair of unorientated univalents in the cytoplasm, or more infrequently still, with chromatid bridges present at first anaphase. However, these were no more frequent than to be observed in most plants of *V. arvensis*.

4. DISCUSSION

From the observations elaborated above it is obvious that not all the AT-intermediates are of recent hybrid origin even though they resemble experimentally produced hybrids between *V. tricolor* and *V. arvensis* in gross morphology. In fact only those plants taken from a mixed population of the two putative parent species were found to be F_1 hybrids. The rest, from populations where at least *V. tricolor* was absent, were of two general sorts, viz. firstly, plants of *V. arvensis* simulating intermediates, and secondly, genetically determined (presumably) AT-intermediates. These two groups are both of interest in the unravelling of the taxonomic problems of the annual pansies, but for different reasons.

The first shows that phenotypic plasticity occurs in populations of *V. arvensis* and may occasionally be sufficient to blur the limits of the species and cause confusion in the naming of plants. Any doubts about such plants, however, should be easily resolved by an examination of the pollen morphology. The second group, on the other hand, raises problems over interpretation and perhaps, incidentally, over the recent evolution of the annual pansies.

On the basis of pollen morphology and cytology—perhaps more reliable indicators

of affinity in this instance than gross floral morphology—the genetically determined AT-intermediates appear most closely allied to *V. arvensis*. This suggests that two possible interpretations of their origin need be considered. Either they arise from past hybridization or there has been confusion over the real limits of *V. arvensis* and they represent extreme variants of this species. Past hybridization may have involved intraspecific or interspecific crosses. The possibility of intraspecific crosses in *V. arvensis* (cf. Clausen 1926), coupled with chromosome instability and loss, being responsible can be eliminated since this would lead to a reduction below $2n = 34$. On the other hand these plants may be 'stabilized' hybrid segregates following interspecific hybridization and subsequent introgression as envisaged by Clausen, but this raises a number of difficulties.

There is no doubt that hybridization between *V. tricolor* and *V. arvensis* does occur for, apart from the F_1 hybrids mentioned here, there are several reports of naturally occurring hybrids by other workers, e.g. Wittrock (1897), Clausen (1922, 1926). The question to be really considered is whether the occurrence of hybridization materially alters the variation patterns of the two species involved. Normally *V. tricolor* and *V. arvensis* do not grow together. Ferdinandsen (1918) and Clausen (1922) have both shown the two species to have their optimal developments on different soil types. *V. tricolor* occurs most frequently on acidic soils, *V. arvensis* on basic soils, with only a slight overlap on neutral to weakly acid soils. This edaphic differentiation was thought by Clausen to be sufficient to prevent hybridization occurring frequently enough to break down entirely the specific limits of the two species.

When the two species hybridize, however, the F_1 is fertile and may produce much seed. Thus it would seem that only a few mixed populations where hybridization occurs would be sufficient to produce a whole range of segregates of intermediate morphology, providing these were not at a marked competitive disadvantage in the population. In the only mixed population seen during the present study F_1 hybrids alone were found. This may have been quite fortuitous as the two species may not have been growing together until the previous year, providing too little time to produce the second generation. However, observations on F_2 and subsequent hybrids grown in the experimental garden showed them to be rather weak and suggest that under field conditions they may be at a distinct disadvantage—an assessment which is borne out by Clausen's observations on similar hybrids.

Even if hybrids can compete successfully in the field introgression will not necessarily occur. The F_1 and F_2 hybrids have been found to be capable of at least some self-pollination and under field conditions it seems likely that they would be largely self-pollinating. Should backcrossing occur it is more likely to be to the *tricolor* parent, which is a fairly well adapted outbreeder, than to the *arvensis* parent which, with its reduced flowers, is virtually always self-pollinating.

As Clausen has already shown, and as has been observed in this study, the chromosome number in successive hybrid generations most frequently reduces from $2n = 30$ of the F_1 towards $2n = 26$ as a result of loss of the extra *arvensis* chromosomes. Yet no AT-intermediates were discovered with these lower numbers. Increase in chromosome number beyond $2n = 34$ through irregular segregation of chromosomes has also been reported by Clausen, and the chromosome number ($2n = 40$) quoted by Fothergill (1944) for *V. contempta* Jord., a yellow-flowered *Tricolores* pansy, could possible be due to this. However, the increase of chromosome number reported by Clausen was of rare occurrence and was associated with chromosome instability. Consequently it is unlikely to have lasting effects on the variation pattern of these pansies.

The production of plants with a stable chromosome number of $2n = 34$ from hybrid segregates requires repeated backcrossing to the *arvensis* parent but this is unlikely to produce AT-intermediates as described above. It is known (Clausen 1926) that a number of inhibitor genes producing reduction of floral parts are present on the extra chromosomes of *V. arvensis*. It is therefore likely that backcrossing to the typical, small-flowered *V. arvensis* would thus produce a small-flowered *arvensis*-type plant rather than the present AT-intermediates.

The interpretation of the AT-intermediates as extreme variants of a rather variable *V. arvensis*, whose exact limits have been confused in the past, raises fewer difficulties. The basis of the differences between *V. tricolor* L. and *V. arvensis* Murr. rests with Murray's reference (Murray 1770) to Haller's distinction (Haller 1745) between the two taxa:

tricolor: 'flore calyce duplo longiore'
arvensis: 'flore calyce paulo majori'

Somewhat incorrectly this distinction has come to be accepted as *V. tricolor* with 'upper petals longer than upper sepals' and *V. arvensis* 'with upper petals shorter, or as long as, upper sepals' (e.g. Clausen 1922, Drabble 1909, 1926, 1927a, 1927b, Warburg 1952). This general difference between the two taxa is associated with their reproductive behaviour. *V. tricolor* is well adapted to cross-pollination and has large, showy flowers with a well-developed stylar flap, whilst *V. arvensis*, as normally understood, is well-adapted to self-pollination and has reduced petals and a very much reduced stylar flap. The distinguishing features of *V. arvensis* are characteristic of successful exploiters of the ephemeral habitat offered by arable farmland, i.e. short life-cycle, increased flower production and reduced flowers promoting self-pollination (cf. Stebbins 1957). It is reasonable to assume, therefore, that *V. arvensis* (as normally understood) has developed from an outbreeding pansy mainly by selection of mutant genes present on the extra *arvensis* chromosomes which inhibit flower development to varying degrees. In view of the marked genetic similarity of the two species, as reflected in the pairing behaviour of the chromosomes in the F_1 hybrid, the original outbreeding *V. arvensis* also would resemble closely the present-day *V. tricolor*.

Therefore one can consider that the morphologically constant, and presumably genetically determined, AT-intermediates, A12/1, A30, A41 and A37, represent either forms closely allied to the original outbreeding *V. arvensis* from which the small-flowered inbreeding *V. arvensis* was developed, or represent the remnants of an intermediate stage between the two. The present variation between populations of the small-flowered *V. arvensis* can therefore be traced back to those differences originally present in the heterozygous, outbreeding progenitors and which have since become segregated out and distributed into the now almost completely inbreeding lines. Such lines are usually morphologically uniform and the presence of the morphologically constant AT-intermediates in these populations must result from the mixing of two separate populations.

On the basis of the available evidence it seems an economy of hypotheses to consider the cytologically regular and non-segregating AT-intermediates as being extreme variants of *V. arvensis* rather than 'stabilized' hybrid segregates. Taxonomically these plants are best considered as representatives of a variable *V. arvensis* Murr. since the observed variation suggests it would be possible to find a complete range in morphology from *V. arvensis*, *sensu stricto*, to the most extreme AT-intermediates defined above.

V. arvensis would then be taken to include: annual pansies with cream, or predominantly cream, never blue or purple, flowers; upper petals usually shorter than, or as long as, the upper sepal, although occasionally longer, up to about $\times 1\frac{3}{4}$; stylar flap usually reduced and appearing absent but sometimes developed enough to be seen with a hand-lens; pollen assemblage composed predominantly of 5-colpate grains with a few 4-colpate grains and occasionally some 6-colpate grains; chromosome number $2n = 34$.

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