Acta Bot. Neerl. 25(1), February 1976, p. 31–40

GENECOLOGICAL INVESTIGATIONS ON ZINC PLANTS
I. Genetics of flower colour in crosses between Viola calaminaria Lej. and its subspecies westfalica (Lej.) Ernst

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SUMMARY

The genetics of flower colour was studied in two subspecies of *Viola calaminaria*. One major gene could account for the "yellow-blue" phenotype, if we assume that inheritance is tetrasomic.

The implications for the origin of the two subspecies are discussed.

1. INTRODUCTION

In northwestern Europe there are two subspecies of the zinc violet *Viola calaminaria*. The nominate subspecies is a yellow flowered plant occurring on abandoned zinc mines between Liége (Belgium) and Aachen (W. Germany); the blue flowered ssp. *westfalica* is restricted to a former lead and zinc mine near Blankenrode (W. Germany).

Morphologically, the most striking difference between these subspecies is the flower colour. The other differences reported in the literature (HEIMANS 1961, ERNST 1965) are small, quantitative and not always maintained in culture.

According to BAUMEISTER (1967) *V. calaminaria* ssp. *westfalica* tolerates somewhat higher zinc levels than *V. calaminaria*.

In view of the different opinions on the origin of zinc tolerant plants and of the zinc violet in particular (see ERNST 1974 for a review) it seemed worthwhile to investigate the genetics of the most conspicuous character: flower colour.

A complex genetic system, leading to a number of intermediate forms would favor the hypothesis of an ancient differentiation of the two subspecies. In contrast, a simple genetic system would suggest a relatively recent origin.

2. MATERIAL AND METHODS

Seeds of *V. calaminaria* were collected near Plombières (Belgium) and of *V. calaminaria* ssp. *westfalica* in the "Bleikühle" near Blankenrode.

Plants used as parents and F1's were grown in the greenhouse, F2's and B1's in the experimental field. Before cross-pollination, the lower petal was removed from the flowerbud to prevent selfing. This method gives far better results than emasculation. Unpollinated controls never set seed, but in the

progeny of cross pollinated plants small numbers of plants that resemble the female parent appear, indicating some rare selffertilisations; these selfings are easily recognised and discarded. Two sets of experiments were run: in the first set, plants of the two subspecies grown from wild seed were crossed. In the second set, the aforementioned plants were selfed for three generations and then crossed.

3. RESULTS

Table 1 summarizes the results of the first set of experiments (the reader may refer to *fig. 1* for the designation of the generations). The phenotype of the parents in this experiment and the following ones is: *V. calaminaria:* flowers purely yellow, with dark violet streaks on the lower petal. *V. calaminaria* ssp. *westfalica:* flowers violet blue, with a yellow spot at the base of the lower petal. The F1 is of the intermediate type in respect to flower colour but rather variable. F2's and B1's give every possible shade between pure yellow and violet-blue. Especially the intermediate types exhibit a great variation. Young flowers of intermediate plants are yellow with a pale blue hue, becoming darker with age. The mature flower is violet-blue except for the lower petal, that is always more or less yellow. Owing to this age-effect nearly all the variation of the family can be observed on one rich flowering plant. The only clear distinction which can be made is between pure yellow flowering plants and those that have at least some blue in the flowers. As can be seen from *table 1* some of the F2's and B1's had small numbers of purely yellow flowering plants. However, the numbers are too small to draw any conclusions. We therefore discuss the results of the

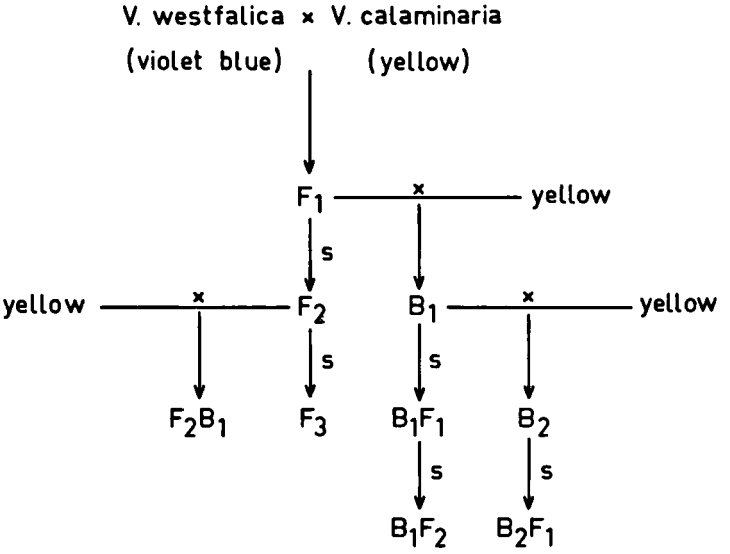

Fig. 1. Scheme of the crosses made and designation of the generations.

Table 1. Results of crosses between plants derived directly from wild populations.

Family	Parent	Generation and type of cross (see *fig. 1*)	Observed numbers			Percentage yellow	95% confidence limits in percents
			yellow	non-yellow	total		
TVC 53	TVB 46a-7	F_2 (west × cal)	1	43	44	1.8	0.08–10.12
TVD 75			0	10	10		
TVC 54	TVB 47b-3	F_2 (west × cal)	2	79	81	2.6	0.75–7.98
TVD 77			1	33	34		
TVD 78	TVB 47b-5	F_2 (west × cal)	0	60	60	0	0 – 5.95
TVC 32	TVB 33-1	F_2 (cal × west)	1	84	85		1.2 – 6.06
TVC 33	TVB 33-2	F_2 (cal × west)	2	31	33	6	0.75–20.08
TVE 92	TVB 33-1	B_1 (cal × west) × cal	0	13	13	0	0 – 9.92
TVF 121			0	22	22		
TVE 93	TVB 33-2	B_1 (cal × west) × cal	0	55	55	1.8	0.31– 6.58
TVF 122			2	56	58		

second set of experiments *(table 2)* first. Here also the number of plants is rather low, so we have included the 95% confidence limits of the percentage of yellow flowering plants. Estimates of this percentage are rather low, with the exception of the families listed in *table 2B*. Selfings of yellow flowering plants always give 100% yellow progeny. Selfings of the blue flowering plants produce sometimes only blue progeny, but in most cases blue and yellow. We conclude that in these crossings "yellow flower" is a recessive trait and the difference "pure yellow" – "coloured" is caused by a small number of genes, possibly one.

As we are dealing with a cross between subspecies we must have, in order to calculate genetic ratio's, information on the meiotic system in the F1's. We have evidence from chromosome observations that multivalent formation is common in the parent subspecies as well as in the F1's. This observation is in accordance with Heimans' view that the zinc violets are tetraploids derived from a 2n = 26 ancestor (HEIMANS 1961). We therefore calculated genetic ratio's for certain boundery cases. We assume that both subspecies are autotetraploids and that there is a one gene difference responsible for the "blue-yellow" phenotypes. In the F1, any "calaminaria" chromosome can pair with one other "calaminaria" chromosome (freq. p) or with a "westfalica" chromosome (freq. q). Under the assumption of random pairing p equals $^1/_2$q (SVED 1966). If there is preferential pairing, we have p$>^1/_2$q.

Multivalents always include a calaminaria-westfalica pair and thus their freq. is a fraction of q (q'). Only quadrivalents are considered in the following computations. Double reduction may occur with a freq. σq'. For q' = 1, σ can have the maximum value of 1/6 (BURNHAM 1962).

If we denote any set of four homologous calaminaria chromosomes as a_c, b_c, c_c, d_c and similarly the westfalica chromosomes as a_w, b_w, c_w, d_w, the gametes of the F1 (taken as $a_c\, b_c\, c_w\, d_w$) can have the following constitution.

Homologous pairs	no pref. pairing no double reduction	double reduction
p	$(1-\sigma)$q	σq'
a_cc_w	a_cb_c	a_ca_c
a_cd_w	a_cc_w	b_cb_c
b_cc_w	a_cd_w	c_wc_w
b_cd_w	b_cc_w	d_wd_w
	b_cd_w	
	c_wd_w	

If both parents were homozygous for the flower colour gene, the F1 will be of the duplex type: AAaa. The following table gives the gametic ratio's and the percentage yellow in F2 and B1 expected in this case.

Table 2. Results of crosses between plants inbred for two generations.

Family	Parent	Generation and type of cross (see *fig. 1*)	Observed numbers			Percentage yellow	95% confidence limits in percents
			yellow	non-yellow	total		
A. Progeny of TVC 9a-2 × TVC 2b-2							
TVE 77	TVD 59a-5	F_2 west × cal	0	10	10	0	0 –30.85
TVE 78	TVD 59b-2	F_2 west × cal	0	57	57	0	0 – 6.25
TVE 98	TVD 59b-2	B_1 west × cal	0	12	12	0	0 –14.04
TVF 124			0	12	12		
TVG 29	TVF 124-4	B_1F_1 west × cal	32	68	100	32	23.04–42.06
B. Progeny of TVC 13a-8 × TVC 2b-2							
TVE 91	TVD 61c-1	F_2 west × cal	2	11	13	16.7	3.61–40.06
TVF 135			1	4	5		
TVF 125	TVD 61c-1	B_1 west × cal	6	4	10	60	26.20– 87.80
TVG 30	TVF 125-2	B_1F_1 west × cal	99	0	99	100	96.38–100
TVG 33	TVF 125-6	B_1F_1 west × cal	94	0	94	100	96.16–100
TVG 31	TVG 125-3	B_1F_1 west × cal	31	67	98	32	22.97– 42.15
TVG 32	TVF 125-4	B_1F_1 west × cal	19	48	67	28	17.89– 40.13
TVG 34	TVF 125-9	B_1F_1 west × cal	38	61	99	38	28.45– 48.29
TVG 35	TVF 125-3	B_2 west × cal	24	16	40	60	46.23– 74.45
TVG 36	TVF 125-4	B_2 west × cal	5	3	8	63	–
TVH 68	TVG 35-1	B_2F_1 west × cal	56	0	56	100	93.64–100
TVH 69	TVG 35-3	B_2F_1 west × cal	17	0	17	100	80.76–100
TVH 70	TVG 35-6	B_2F_1 west × cal	18	0	18	100	81.83–100
TVH 71	TVG 35-8	B_2F_1 west × cal	92	0	92	100	96.08–100
TVH 74	TVG 35-17	B_2F_1 west × cal	9	0	9	100	71.01–100
TVH 77	TVG 35-20	B_2F_1 west × cal	20	0	20	100	83.15–100
TVH 73	TVG 35-16	B_2F_1 west × cal	3	9	12	25	5.56– 54.33
TVH 78	TVG 35-31	B_2F_1 west × cal	0	10	10	0	0 – 30.85
TVH 63	TVG 31-14	B_1F_2 west × cal	11	0	11	100	71.95–100
TVH 64	TVG 31-18	B_1F_2 west × cal	49	0	49	100	92.92–100
TVH 66	TVG 31-32	B_1F_2 west × cal	10	0	10	100	69.15–100
TVH 67	TVG 31-33	B_1F_2 west × cal	59	0	59	100	93.95–100

Family	Parent	Generation and type of cross (see *fig. 1*)	Observed numbers			Percentage yellow	95% confidence limits in percents
			yellow	non-yellow	total		
C. Progeny of TVC 2b-2 × TVC 13a-8							
TVE 67	TVD 58a-1	F_2 cal × west	3	13	16	18	7.83– 33.39
TVF 111			4	18	22		
TVE 68	TVD 58a-2	F_2 cal × west	11	79	90	16	10.09– 23.64
TVF 112			8	24	32		
TVE 69	TVD 58a-3	F_2 cal × west	0	22	22	0	0 – 7.89
TVF 113			0	22	22		
TVE 70	TVD 58a-4	F_2 cal × west	0	81	81	0	0 – 2.41
TVF 114			0	70	70		
TVE 71	TVD 58a-5	F_2 cal × west	0	22	22	0	0 – 15.32
TVE 72	TVD 58a-6	F_2 cal × west	4	41	45	10	9.79– 21.66
TVF 116			11	94	105		
TVE 73	TVD 58a-7	F_2 cal × west	0	31	31	0	0 – 5.58
TVF 117			0	33	33		
TVE 74	TVD 58b-1	F_2 cal × west	4	44	48	7	2.36– 15.70
TVF 118			1	20	21		
TVE 75	TVD 58c-1	F_2 cal × west	7	72	79	12	6.76– 19.32
TVF 119			7	28	35		
TVE 76	TVD 58c-2	F_2 cal × west	6	29	35	17	6.60– 33.05
TVE 96	TVD 58a-3	B_1 cal × west	0	19	19	0	0 – 17.21
TVE 97	TVD 58c-2	B_1 cal × west	3	4	7	43	–
TVG 25	TVF 112-1	F_3 cal × west	0	19	19	0	0 – 17.21
TVG 26	TVF 112-16	F_3 cal × west	0	112	112	0	0 – 3.24
TVG 27	TVF 112-22	F_3 cal × west	19	74	93	20	12.46– 29.53
TVG 28	TVF 112-31	F_3 cal × west	98	0	98	100	96.31–100

	gametes			% yellow in B1	% yellow in F2
	AA	Aa	aa		
100% pref. pairing	0	1	0	0	0
no pref. pairing no double reduction	1	4	1	16.7	2.8
100% multivalent max. double red.	2	5	2	22.2	4.9

If the westfalica-parent was triplex for the flower colour gene (AAAa) we would expect in the F1 also plants of the simplex type (Aaaa) that would give the following ratio's:

	gametes			% yellow in B1	% yellow in F2
	AA	Aa	aa		
100% pref. pairing	0	1	1	50	25
no pref. pairing no double red.	0	1	1	50	25
100% multivalent max. double red.	1	10	13	54	29

Inspection of *table 1* and the left part of *fig. 2* reveals that the two B1's and five F2's examined have percentages of yellow significantly lower than 50 resp. 25. Thus the genotype of the five F1 plants tested was presumably AAaa. Therefore the only conclusion that can be drawn from these experiments is that the three westfalica parents used in these crosses must have been of the quadruplex or triplex type.

In *table 2A* family TVG 29 has about a quarter yellow flowering plants. The lower confidence limit is distinctly higher than 4,9%. So TVG 124–4 was presumably Aaaa.

In *table 2B* we see that all the families showing any segregation fall into the "simplex scheme". The results strongly suggest that TVD 61c–1 was Aaaa.

The backcross TVF 125 consisted of 10 plants, five of which were selfed and two were also backcrossed. The two yellow plants produced only yellow progeny, whereas the three blue ones showed a 3 : 1 or 1 : : 1 segregation.

In most families there was a slight excess of yellow flowering plants. As this excess could be caused by the false inclusion of very light Aaaa plants in the "yellow" group, ten yellow plants were selfed. All the progenies showed 100% yellow flowering plants.

Our conclusion is that classification was correct and that the excess of "yellow" is caused by some factor favouring "a" gametes or zygotes.

The *table 2C* summarizes the results of one family. Ten F1 plants were selfed. Of these, four must have been of the duplex type and two of the simplex type,

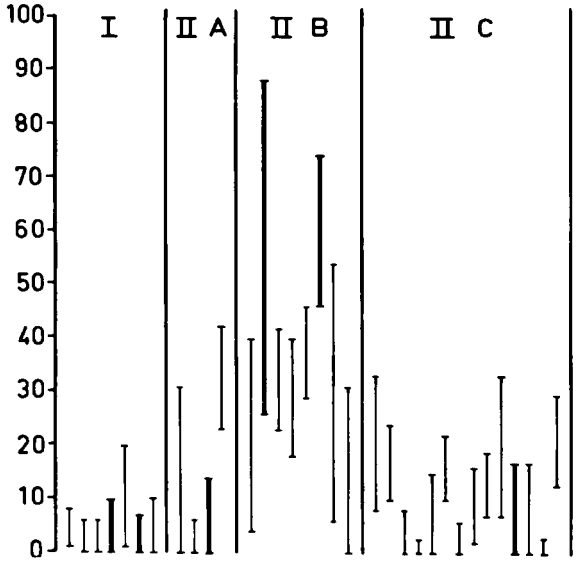

Fig. 2. Summary of the 95% confidence limits of tables I and II. Heavy lines designate backcross results.

whereas the progenies of four plants gave intermediate percentages, probably due to sample errors. We conclude that the westfalica parent used in this experiment was of the triplex type. From the F2 of plant TVD 58a–2, that gave a good 1 : 3 segregation in the second year, four plants were selfed to give F3's (TVG 25–28). Of these two blue plants did not produce yellow, one blue plant gave a clear 1 : 3 segregation and the fourth one, which was yellow, produced only yellow flowering plants.

4. DISCUSSION

If we review the results of eight years of experimentation, it appears that the easiest way to explain our findings is to assume a one locus difference, the westfalica plants tested being either of the quadruplex or the triplex type. The chance of finding a yellow flowering plant in a population mainly consisting of AAAA and AAAa plants must be very low, even close to zero if we assume a considerable amount of preferential pairing. Nevertheless we expected to find at least some yellow flowering plants among several thousands examined in the field. No such plant was ever found. A possible explanation is that plants heterozygous for the flower colour gene arise by introgression and that there is strong selection against yellow flowering segregants (KAKES 1973).

In all crossings described, the fertility is very low, (0.5–2.1 plant/pollination). These figures are, however, not significantly lower than those for comparable crossings within the two species.

The results do not indicate a reproductive barrier, but as CLAUSEN (1931) already demonstrated this is not related to species differentiation in the section *Melanium* of the genus *Viola*.

The consistency of our results proves that in the F1 as well as in later generations there is a regular segregation. Apparently preferential pairing is very limited. This together with the apparently simple genetic system underlying the difference in flower colour, supports the hypothesis of a close relationship between the two subspecies. ERNST (1974) suggests that the zinc violets arose recently (i.e. after the glacial) from *V. tricolor*. If this were true, *V. tricolor* and *V. calaminaria* must once have occupied adjacent sites. The two species, however, exhibit rather big differences in their ecological preference. *V. tricolor* is found in open vegetation on soils with low calcium content, whereas *V. calaminaria* only occurs on zinc mines with high calcium content and in a much more dense vegetation of other zinc tolerant plants. This is not to say that *V. calaminaria* is a calcicole plant, as we know that Zn toxicity is strongly influenced by the calcium content of the soil. (JOWETT 1964) Today *V. calaminaria* and *V. tricolor* nowhere occur in close vicinity. The only heavy metal rich area where we have found *V. tricolor* is on the zinc and copper rich shales of Werdern-Ramsbeck (W. Germany), where it is common on the mine tips. In this area however, *V. calaminaria* is absent. Schultz's hypothesis on the origin of zinc plants (SCHULTZ 1912), extended by Heimans to the zinc violets seems to us the most probable one. The two subspecies, then, once must have been specialized parts (on natural ore outcrops) of a more continuous population of violets in the late glacial that was broken up by disappearance of the non tolerant ecotypes in the warmer periods that followed.

ACKNOWLEDGEMENTS

We thank Dr. Sybenga for carefully reading the manuscript and offering valuable suggestions.

The technical assistance of E.T. J. M. Anink and H. Bos is gratefully acknowledged.

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