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GENECOLOGICAL INVESTIGATIONS ON ZINC PLANTS
V. BARRIERS TO GENE FLOW LIMITING THE INTROGRESSION OF VIOLA ARVENSIS (MURR.) INTO VIOLA CALAMINARIA LEJ. SSP. WESTFALICA (LEJ.) ERNST

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SUMMARY

Hybrids of *V. arvensis* (annual) and *V. calaminaria* (perennial) are regularly found on an open pit mine near Blankenrode (W. Germany). Morphological as well as karyological studies show that these hybrids represent different generations of selfing and/or backcrossing to *V. calaminaria.* The hybrids show a low tolerance to zinc.

To study the gene flow the reproductive strategy of the parent species, their F_1 and F_2 hybrids was compared in a normal soil. The progeny of natural hybrids with *V. calaminaria* was investigated under zinc stress. The results show that F_1 and F_2 hybrids have a higher fitness than *V. calaminaria* if both are grown in normal soil. This is caused by hybrid vigour and by a combination of the reproductive strategies of the parent species. When the progenies of hybrid plants are cultivated together with *V. calaminaria* in soil with zinc added to it, no difference in reproductive capacity can be shown. Prolonged observations in the field have shown that the longevity of established hybrid plants is only slightly less than that of non-hybrids.

The observations are in accord with the assumption that a dynamic equilibrium exists between gene flow and selection. The consequences of this assumption for the genetic variation in *V. calaminaria* are discussed.

1. INTRODUCTION

V. calaminaria ssp. westfalica, the blue flowered zinc violet, is an endemic species occurring on an abandoned zinc mine and a zinc rich meadow near the village of Blankenrode (W. Germany). The number of individuals in the area is less than 6000 whilst the number of plants substantially contributing to the gene pool may be less than 1000. It is presumed that the zinc violets have been isolated since the last glacial period (SCHULTZ 1912, HEIMANS 1961). Their limited distribution, even within the zinc rich area, points to the fact that strong selective forces are operating on these violets. From a theoretical point of view one would expect a small amount of genetic variation in such an endemic species (STEBBINS 1942, NEI 1975). However, the contrary is true. There is considerable genetic variation in morphological characters as well as in zinc tolerance. It has been shown in an earlier paper (KAKES 1977) that introgression from *V. arvensis* influences the gene pool of *V. calaminaria ssp. westfalica.* The low tolerance to zinc of most of the natural hybrids prevents their establishment in the greater part of the area where the zinc violets occur (KAKES 1981).

As pointed out by ANDERSON (1949) and HEISER (1973) introgression not only depends on the formation of a primary hybrid, but also on the reproductive capacity of later stages in the hybridisation. In this case the two parent species have completely different life strategies. *V. arvensis* is an annual pioneer weed, whilst *V. calaminaria ssp. westfalica* is a slow growing perennial, normally occurring in dense vegetation.

To estimate the amount of gene flow, and potential reproductive capacity, the reproductive effort (HARPER & OGDEN 1970) of *V. arvensis, V. calaminaria ssp. westfalica,* and their F_1 and F_2 hybrids was compared without zinc stress. The reproductive effort of *V. calaminaria ssp. westfalica* and natural hybrids was compared under zinc stress.

2. MATERIAL AND METHODS

Random seed samples of *V. calaminaria ssp. westfalica, V. arvensis,* their F_1 and F_2 hybrids were sown in the greenhouse of the department in February and planted out in the experimental garden in April 1978 (see also KAKES 1981). From the start of flowering till the end of August all open flowers of 10 plants of each family were counted and the ripe capsules were collected. The first 50 closed and ripe capsules of each plant were harvested separately. In the first week of September the shoots were harvested. The dry weight of capsules and shoots was determined after 1 hr at 105°C and 48 hrs at ambient temperature and humidity.

The seed content of the open dehisced capsules was estimated by determining the ratio: weight of seeds/weight of pericarp.

The experiments with zinc stress were performed with garden soil to which different quantities of zinc were added in the form of zinc sulphate, zinc acetate, and zinc-rich slag material. The mixtures were made in 1975 and put into concrete containers (cold frames) in a 10 cm deep layer. At the time of the experiment (summer 1979) the soil had a closed plant cover, mainly consisting of zinc-tolerant *Agrostis tenuis*. The available zinc levels of the different plots were determined at the start of the experiment. Available zinc was estimated by the method of KAKES (1981).

3. RESULTS

The differences between the groups of plants were clear from the first weeks. *V. arvensis,* a species with a plastic phenotype grew to enormous dimensions under the favourable conditions in the experimental garden. However, the F_1 "west. × arv." soon surpassed both parents. *Fig.* 1 shows the cumulative number of flowers produced from May to August 1978. The F_1 flowers earlier and more profusely than *V. calaminaria ssp. westfalica.* The difference in the

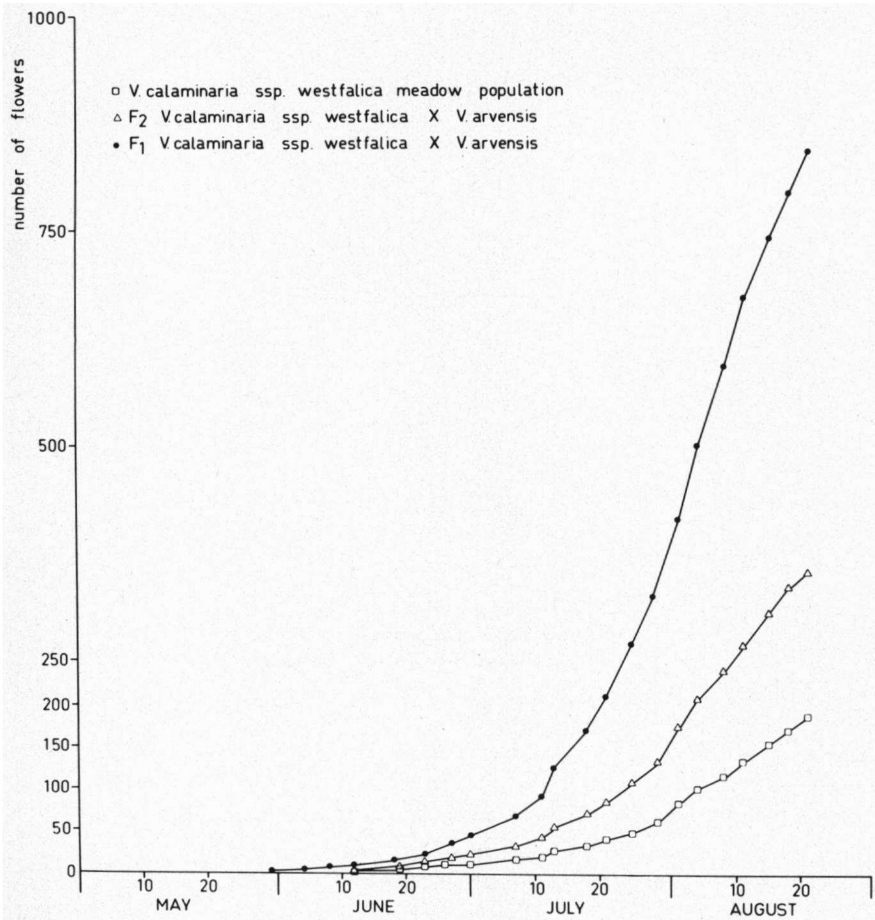

Fig. 1. Cumulative graph of the number of flowers produced by three families of violets.

number of flowers produced became significant ($p < 0.025$) in the last week of June. The F_2 behaved very erratically, some plants produced as many flowers as the F_1 but others did not flower at all. As a consequence the mean for the F_2 was intermediate between *V. calaminaria ssp. westfalica* and the F_1, with a large variance. In August the difference between all groups was significant ($p < 0.025$). *Fig. 2* shows the seed production of the three groups of plants. The differences are even greater than those in flower production. There are two reasons for this: Firstly the weight per capsule is greater in the F_1 and secondly *V. calaminaria ssp. westfalica* virtually stops producing seeds after August 10th. However, the latter phenomenon may be an artefact, caused by the unnatural growing conditions. Again, the F_2 was very variable, three plants were sterile, whilst the seed production of the other seven ranged from 0.3 g to 16.8 g per individual. *Table 1* shows the dry weight of shoots harvested in September. The

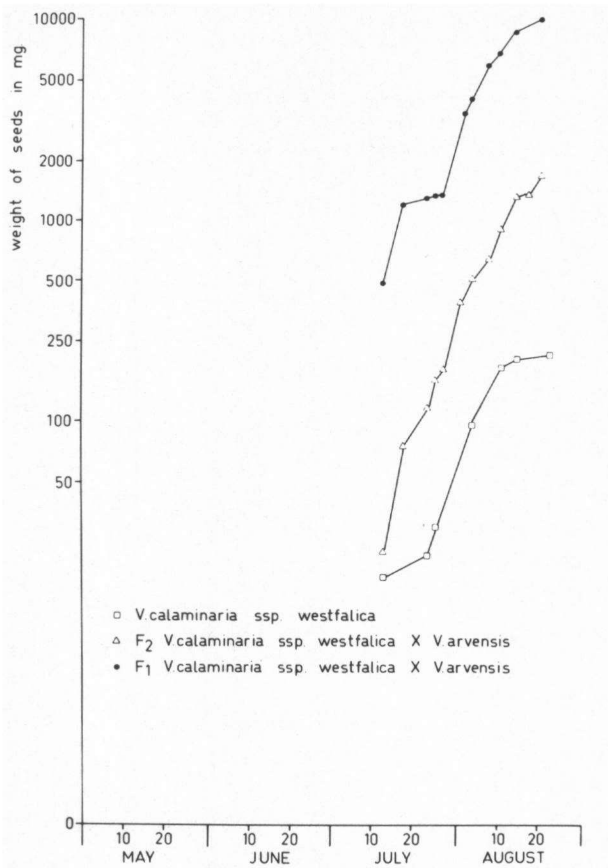

Fig. 2. Cumulative graph of the amount of seed produced by three families of violets.

two populations of *V. calaminaria ssp. westfalica* were not statistically different. The F_1 is different from *V. calaminaria ssp. westfalica* but not from *V. arvensis*. The F_2 has an intermediate dry weight and a large variance, due to the occurrence of some extremely slow growing plants, as well as plants growing as well as the F_1. The difference in vegetative growth would be of little importance if the hybrids had the annual life cycle characteristic of *V. arvensis*. However, *table 2* shows that this is not the case. Of the F_1 plants 74% survived the relatively mild winter of 1976/1977, not significantly less than *V. calaminaria ssp. westfalica*. Of the F_2, only 30% survived, which is not unexpected, as this generation has a high proportion of apparently less well adapted genotypes.

Whereas the results clearly show that the potential reproductive capacity of hybrids is higher than that of *V. calaminaria ssp. westfalica* they do not show that the fitness of these hybrids is higher in the natural habitat. Ideally the experiment should be repeated in the field but this is not possible since, among other things, part of the natural population would have to be destroyed, including

Table 1. Dry weight of the shoots of *V. calaminaria ssp. westfalica, V. arvensis* and hybrids between *V. calaminaria ssp. westfalica* and *V. arvensis.*

Population/family	*n*	*dry weight in grams*	*l.s.d.*
V. calaminaria ssp. westfalica meadow population	9	18.6	± 5.2
V. calaminaria ssp. westfalica mine population	9	23.1	± 9.0
V. arvensis	5	71.4	±27.1
F_1 *V. calaminaria ssp. westfalica* × *V. arvensis*	7	75.9	±16.4
F_2 *V. calaminaria ssp. westfalica* × *V. arvensis*	10	43.8	±19.7

Statistics (one way analysis of variance)

Comparison	F value	P
All populations/families	$F^4_{35} = 14.44$	$< 10^{-3}$
Meadow pop./mine pop.	$F^1_{16} = 1.00$	1.00
V. calaminaria ssp. westfalica/F_1	$F^1_{23} = 98.63$	$< 10^{-3}$
V. calaminaria ssp. westfalica/F_2	$F^1_{27} = 10.18$	0.004
V. arvensis/F_1	$F^1_{10} = 0.16$	0.7
V. arvensis/F_2	$F^1_{13} = 3.68$	0.08

Table 2. survival of *V. calaminaria ssp. westfalica* and its hybrids with *V. arvensis.*

Population/Family	*number of plants*	*number of survivors*	*% survival*
V. calaminaria ssp. westfalica	196	161	82.1
F_1 *V. calaminaria ssp. westfalica* × *V. arvensis*	156	116	74.4
F_2 *V. calaminaria ssp. westfalica* × *V. arvensis*	564	168	29.8

Statistics

Comparison	χ^2	df	P
All families	68.78	2	$< 10^{-3}$
V. calaminaria ssp. westfalica/F_1	0.38	1	0.54
V. calaminaria ssp. westfalica/F_2	55.83	1	$< 10^{-3}$

a number of the comparatively rare hybrids. We have circumvented this problem in two ways. Firstly we have, from 1971 on, marked and described hybrid and non hybrid plants, to estimate their longevity. *Table 3* shows the results of these observations. The difference between the two groups is not significant. The error is large, however, because the plants were observed only once or twice a year, and owing to the distribution of the data, any statistical treatment is bound to give a loss of information. Secondly, the progenies of hybrid plants were grown in plots with different zinc levels, together with plants from random seed samples of *V. calaminaria ssp. westfalica. Table 4* shows the results. At 1.8 ppm Zn the vegetative and seed production of hybrids is significantly higher ($p < 0.001$) than that of non-hybrids. At 8.0 and 296 ppm there are no significant

Table 3. Minimal age of individual plants in the Bleikuhle at Blankenrode.

years	hybrids	non-hybrids
1	23	17
2	5	8
3	5	6
4	7	3
5	2	7
6	0	1
7	1	2
8	0	1

Table 4. Dry weight (in mg) of hybrid and non hybrid plants in plots with different soil concentration of available zinc.

A shoots: Type of plants	Available Zn level in ppm 0.5	1.8		8.0		296			
Non hybrids	205 ± 20	298 ± 34	***	394 ± 101	N.S.	297 ± 48	N.S.		
Hybrids	–	650 ± 120		270 ± 25		301 ± 25			
B seeds:									
Non hybrids	30 ± 20	35 ± 14	***	32 ± 51	N.S.	53 ± 42	N.S.	N.S	N.S.
Hybrids	–	189 ± 112		49 ± 18		† 91 ± 45			
						†260 ± 273	**		
						† 58 ± 19			

† Three different progenies.
Significance of differences: * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; N.S. $p < 0,05$.

differences between hybrids and non-hybrids. There are, however, significant differences in seed production ($p < 0.01$) between different hybrid groups, especially at the highest zinc concentration. As these families were derived from different natural hybrids, this effect is probably due to the variation in zinc tolerance among these natural hybrids as found by KAKES (1981).

4. DISCUSSION

Hybrids between two species with a very different reproductive strategy have been examined. *V. arvensis* is a pioneer species of arable land, predominantly inbreeding, strictly annual, and has great phenotypic plasticity. Under unfavourable conditions small plants are formed which produce one or two capsules and die thereafter, whereas under favourable conditions large plants are formed which produce many capsules and as much as 10 g of seed per individual per season. *V. arvensis* is a typical r-species (HARPER 1977). On the other hand *V.*

calaminaria ssp. westfalica is a perennial species, mostly occurring in dense vegetation and almost exclusively outbreeding. Its phenotypic plasticity is not as great as that of *V. arvensis,* but it can survive many years without producing seed, reproducing vegetatively by stolons. *V. calaminaria ssp. westfalica* may be well called a K-species.

It is thus possible to study natural hybrids between an r-species and a K-species. The results show, that hybrids combine the strategies of their parents in a way that is very favourable for their survival and reproduction. They tend to produce more seeds, but they also form stolons, and a large proportion of the hybrids are perennial. The difference in reproductive strategy becomes particularly clear if we compare the ratio: weight of seeds/total weight of plants for the two parental species and their F_1 and F_2 hybrid derivatives. This ratio is 0.06 for *V. calaminaria ssp. westfalica,* 0.24 for the F_1 "west. × arv." and 0.11 for the F_2. The F_1 has the additional advantage of a marked hybrid vigour and reproduces mainly by self-fertilisation. Independence from insect visitors can be an advantage in plants that are extremely heterozygous. However, there are a number of factors lowering the fitness of hybrids. Their low tolerance to zinc (Kakes 1981) inhibits their growth even at moderate concentrations of zinc in the soil. In the F_2 unbalanced genotypes are frequent. The seed fertility of natural hybrids is approximately 50% of that of non-hybrids, and slow growing and sterile or semisterile individuals form about 25% of an artificial F_2. There is also some evidence that the competitive ability of hybrids is lower than that of *V. calaminaria ssp. westfalica.* These factors together suggest that hybrids between *V. arvensis* and *V. calaminaria ssp. westfalica* can only establish themselves in boundary situations with comparatively low levels of available zinc, especially in those situations where the plant cover is disturbed by ploughing or digging.

The dynamic equilibrium between gene flow and selection, if it exists at all, is a very delicate one. Any disturbance, especially in areas with low zinc levels can be advantageous for hybrid plants and cause the breakdown of the well adapted genotype of *V. calaminaria ssp. westfalica.*

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