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CYTOGENETIC AND BIOLOGICAL STUDIES IN *GERANIUM ROBERTIANUM* L.

BY

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1. INTRODUCTION

The present work is a continuation of similar publications dealing with *Prunella vulgaris*, *Plantago lanceolata*, and *Veronica officinalis*. The purpose of these investigations amongst others is that of providing a material which may be used later in treatments of general biological or evolutionary problems. They may further be apprehended as contributions to or preliminary studies of a Flora biologica danica, a work which for each species should include both cytogenetic and ecological data. An interesting attempt at a flora of this kind is being published in the series Biological Flora of the British Isles in the Journal of Ecology. Here, however, the cytogenetic aspect of the investigations is not particularly emphasized, see e.g. CLAPHAM, PEARSALL, and RICHARDS (1942). The importance of including the cytogenetic material, however, is very great; for the study of the variation, chromosome number, etc., of the various species, offers a solid basis for everybody who wants to study the species in question. Both plant geographers, students of vegetation, pollen analysts, physiologists, and taxonomists base a very large number of their conclusions on conditions in the individual species, and this is often done without the researcher in question having made himself acquainted with the width of variation and the genetic conditions of the species. Only when a cytogenetic analysis of the species has been carried through, this is complelely serviceable as material in other kinds of investigation.

The species *Geranium Robertianum* L. has been thoroughly treated by KNUTH (1912), EVANS (1920), GAMS (1924), and WANGERIN (1926). The various attempts at classifying the large number of varieties show pronounced mutual discrepancies (cf. ROUY & FOUCAUD, Flore de France, KNUTH, and EVANS).

Cultivations were made by GRAEBNER (Syn. d. mitteleurop. Flora VII, 63, 1913—14), OSTENFELD (1920), and EVANS (1920). From these cultivations it seems evident that the collective species *G. Robertianum* includes several distinct subspecies, viz. the ssp. *purpureum* Vill. and *celticum* Ostf.

Cultivation experiments have been made by the present writer in the Botanical Gardens of the University of Copenhagen. Three cultivation experiments were made, viz. in 1937—38, 1940—41, and 1942—43. They were all planned as varied environment experiments. In 1943—45 the material was supplemented by cytological observations and by a single crossing experiment. Particular importance was attached to a comparison between *G. Robertianum* var. *genuinum* Gren. & Gordr. and var. *rubricaule* Hornemann (in WILK. & LANGE, Prodr. Fl. Hisp. 1878). The latter is found in the shingle of stony beaches. Thus *G. Robertianum* occupies two ecologically widely different localities, viz. the shaded, sheltered, and moist woods and the sunny, windswept shingle beaches. It has been discussed whether var. *rubricaule* is a modification, an ecotype, or a combination of both. OSTENFELD (*loc. cit.* p. 552) states that "*G. Robertianum* from sunny localities is usually very red all over (f. *rubricaule*)", and the accounts of ROUY, GAMS, and WANGERIN prove that these writers, too, consider var. *rubricaule* as a modification. The typical and original var. *rubricaule*, however, came from a stony seashore (according to EVANS it is identical with var. *littorale* Rouy and var. *maritimum* Babington) and differs in growth habit from the var. *genuinum* of sunny exposures (e. g. screes and clearings).

2. Chromosome Countings.

Cytological investigations of *G. Robertianum* s. l. have been made by GAUGER (1937) and WARBURG (1938). GAUGER in material from Antwerp, Trieste, Schleswig-Holsten, and Kiel found n = 28, while WARBURG in *G. Robertianum* from Cambridge and the ssp. *purpureum* from Great Britain and Montpellier in France found n = 16. The present writer has studied somatic mitosis in root tips of nine of the cultivated types. The results of these countings may be summarized as follows:

No. 455.	Ssp. *purpureum*	Bot. Gardens, Lisbon	2 n = 32 (Fig. 1).
No. 452.	Var. *genuinum*	Harager Hegn (wood, Sealand)	2 n = 64
No. 453.	—	Bot. Gardens, Nancy	2 n = 64
No. 454.	—	Bot. Gardens, Ghent	2 n = 64 (Fig. 1).
No. 447.	—	Bot. Gardens, Cracow	2 n = 64 (Fig. 1).
No. 448.	—	Bot. Gardens, Cluj	2 n = 64
No. 467.	—	Bot. Gardens, Leyden	2 n = 64 (Fig. 1).
No. 450.	Var. *rubricaule*	Klintebjerg, Sealand	2 n = 64
No. 451.	—	Lille Vrøj, Sealand	2 n = 64 (Fig. 1).

Fig. 1. Somatic metaphase plates from root tips. 2 n = 32 (No. 455) and 64 (The other numbers).

Warburg's figure for ssp. *purpureum* could be corroborated. But no plants were found with the figure 2 n = 56, a figure which, for that matter, disagrees highly with 32. I doubt the correctness of 2 n = 56, in particular after being taught by experience how easily a less felicitous technique of staining may be misleading. Only after several more or less unsuccessful attempts did I succeed in preparing a material in which the figure 2 n = 64 could be established. The roots were fixed in Nawashin's fluid and were stained by means of Newton's iodine gentian violet technique. The favourable metaphase plates, on which the cromosomes were sufficiently separated, however,

could only be stained after the slides were hydrolysed in hydrochloric acid as in the FEULGEN staining, or if a very long period of staining was used (24 hours or more), which permitted a prolonged differentiation in alcohol and cloves oil.

Many of the chromosomes are provided with median or submedian constrictions. In no. 454 trabants could be observed in two of the chromosomes. It proved impossible to ascertain chromosome-morphological differences between the examined types with 64 chromosomes.

As WARBURG also gives the figure 2 n = 32 for var. *genuinum* of *G. Robertianum*, it thus seems that there are dibasic and tetrabasic races of this species. According to investigations published it is, however, evident that the British *G. Robertianum* population is in need of renewed investigations.

3. Cultivation Experiments.

The seed samples were collected in nature. Only in the experiments 1942—43 some additional samples from various botanical gardens were used. In the experiments 1937—38 and 1942—43 the plants were kept in pots, while in 1940—41 the seedlings were planted in boxes or in the experimental field. As only a limited space was available for making experiments, particularly under different exterior conditions, the number of experimental plants of the same cultivation number under the same exterior conditions was kept at 8 or 10. So the measured values of the height, breadth, etc., are not suitable for statistical treatment. However, in order to give some impression of the width of variation, I shall besides the mean numbers also give the extreme limits for the variation of the values measured. As appears from the photographs, the differences treated here are as a rule so great that it would only in a few cases be of any use to support one's conclusions on calculations of mean errors.

Cultivation 1937—38. After germination (April 1937) the seedlings of the two cultivated types appeared to be different, the seedlings of var. *rubricaule* (No. 62, shingle beach at Maarup,

Fig. 2. Cultivation 1937—38. Plants cultivated in the shade: Var. *rubricaule* (No. 62) on the left, without flowers; var. *genuinum* (No. 147) on the right, flowering. B. phot. May 27, 1938.

the Isle of Samsø) being less hirsute than those of var. *genuinum* (No. 147, wood near Holte). None of the plants flowered in the first year, and hibernation took place in a typical rosette stage. All plants died in the late autumn of 1938.

Fig. 3. Cultivation 1940—41. Two of the plants from the wood-soil boxes. Var. *rubricaule* on the left and var. *genuinum* on the right. B. phot. August 22, 1941.

Table 1.

Field No.	Character	Date 1938	I. Shaded		II. Unshaded	
			Mean	Range	Mean	Range
62 var. *rubricaule*	Number of flowers per individual	May 27th Sept. 28th	0.0		1.1	0—3
	Height in cm.....		17.2	10—23	13.5	8—19
	Breadth in cm	—	42.3	30—56	33.5	26—48
	Breadth:Height	—	2.5		2.5	
147 var. *genuinum*	Number of flowers per individual	May 27th Sept. 28th	10.3	1—24	13.3	6—33
	Height in cm.....		24.7	18—33	27.5	23—35
	Breadth in cm	—	25.0	19—36	25.8	22—29
	Breadth:Height	—	1.0		0.9	

The 20 individuals of the two types were divided into two parts and kept in two frames, one of which was shaded. In the first summer only small differences were noticed, but in the second summer the illuminated plants of the two types became widely different, var. *rubricaule* being late-flowering and of low growth, var. *genuinum* being early and large (Fig. 2). Exposure to light seemed to promote flowering and (in the case of var. *rubricaule*) to retard the growth (cf. Table 1).

Cultivation 1940—41. In order to study the influence of different soil conditions, another varied-environment experiment was started. Seeds of *rubricaule* plants from the stony beach below Klintebjerg in Odsherred (No. 256) and *genuinum* plants from the wood Ermelunden near Copenhagen (No. 269) were sown, and in May 1940 the seedlings were planted in the field. 24 plants of each type were set out, 8 individuals being planted in two boxes filled with shingle, gravel, and sand, 8 plants in two boxes filled with a somewhat clayey field soil covered with a 5—8 cm layer of withered or decayed leaves from a wood, and 8 individuals in the experimental field in the shade (illumination 18—20 per cent. of that of the open air). Each box was 40 cm deep. The boxes were sunk in the experimental field.

The year 1940: in contrast to the culture in pots from 1937—38,

Table 2.

Field No.	Character		Date Year	I. Shaded		II. Unshaded			
						a. Wood soil		b. Shingle	
				Mean	Range	Mean	Range	Mean	Range
256. var. *rubricaule*	Length of longest rosette leaf stalks in cm		Sept. 24th 1940	16.3	15—18	12.4	10—15	6.6	5—8
	Height in cm		Aug. 22nd 1941	12.3	10—14	13.8	10—16	11.0	10—13
	Breadth in cm		—	57.8	56—62	43.0	36—50	47.0	45—50
	Breadth : Height		—	4.7		3.1		4.3	
	Dry stuff weight per individual in grams.	Stems +leaves	—	18.9	17.1—21.2	30.6	25—33	5.9	3.2—8.6
		Roots		0.6	0.4—0.7	6.0	5.7—6.4	2.3	1.4—3.2
	Dry stuff weight stems+leaves : roots		—	31.5		5.1		2.6	
269. var. *genuinum*	Length of longest rosette leaf stalks in cm		Sept. 24th 1940	21.0	19—23	23.3	20—30	14.4	10—22
	Height in cm		Aug. 22nd 1941	42.3	37—45	41.0	35—47	27.0	25—29
	Breadth in cm		—	67.3	63—70	70.3	67—74	48.3	30—50
	Breadth : Height		—	1.6		1.7		1.8	
	Dry stuff weight per individual in grams.	Stems +leaves	—	66.8	22—120[1]	42.0	23—51	4.6	2.9—8.2
		Roots		2.1	1.6—2.5	16.0	14.5—18.1	2.6	1.7—4.5
	Dry stuff weight stems+leaves : roots		—	31.8		2.6		1.8	

[1] Two of the individuals were crushed between the others, hence the wide range.

this culture exhibits a difference between the two types already during the first summer; both rosettes and leaves were larger in var. *genuinum* (Table 2). In June all plants cultivated on shingle were somewhat reddish; in September all illuminated plants were yellowish green or more or less reddish, while the plants cultivated in the shade were green. The *rubricaule* plants were more prostrate when cultivated on shingle.

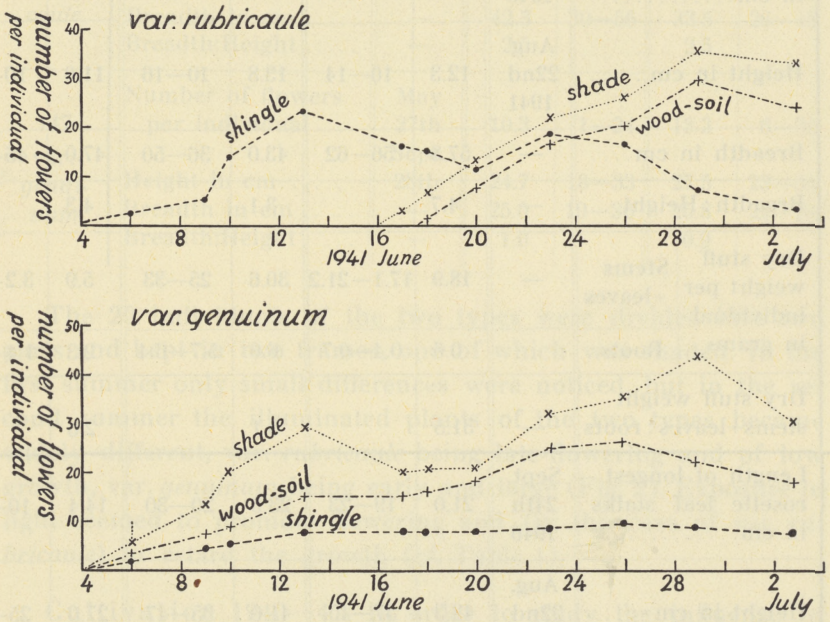

Fig. 4. Flowering of *Geranium Robertianum* cultivated in the shade, in wood-soil, and in shingle. The decrease in number of flowers after the 13th of June seemed to be a function of climatic factors. In most cases the number of flowers follows the size of the plants (cf. Table 2).

The year 1941: As appears from Table 2, the difference between the values of the ratio breadth: height is much greater in this material than in the material from 1937—38, where the plants were kept in pots.

The dry weight is greatest in var. *genuinum*, and among the *genuinum* plants the shade plants are largest. On the other hand the illuminated wood-soil plants have the greatest production of dry stuff in var. *rubricaule*.

The earliness and flowering were studied by counting the flowers per individual (Fig. 4). The difference between the types

with respect to earliness is very pronounced in the shade and wood-soil cultures, while all plants in the shingle boxes flowered at the same time. Among other differences not recorded in the table the following should be noted: (1) the size of the leaves, the largest leaves of var. *rubricaule* being 2.5—5 cm. long, those of var. *genuinum* 4—10 cm.; (2) diameter of flowers: var. *rubricaule* 1.14 cm. (mean), var. *genuinum* 1.25 cm. This difference was not noticed in the material from 1937—38.

In nature *rubricaule* plants generally have a very long root, which is only sligthly branched in the upper part of the soil (between the stones), while it is considerably branched where the sand or gravel below the stones contains some humus (e. g. from decayed algae). On the other hand the *genuinum* plants in woods have short roots, which ramify most frequently in the uppermost soil-layer rich in humus. This difference between the types to a very large extent is due to environmental factors (shade and light, see Table 2). The experiments, however, seem to show that the roots of the *rubricaule* plants cultivated in wood-soil tend to branch out in a deeper layer of soil than the corresponding *genuinum* plants. Furthermore, the ratio stems + leaves : roots gives higher values in the *rubricaule* plants when these are cultivated in the sun.

Analogously the red colour of *G. Robertianum* from sunny exposures is largely due to the influence of the environment. In June 1941 the shade and wood-soil plants were green, while the shingle plants were reddish. In September all unshaded plants were more or less red and even the shaded plants of var. *rubricaule* appeared somewhat reddish, which indicates a greater predisposition to red colouring in this variety. The name of *rubricaule* is somehwat misleading, since the contents of anthocyanin to a fairly high degree depend on the intensity of illumination and to some extent on the quality of the soil. On genetic factors in connexion with the reddish colour see below pp. 21—22.

Cultivation 1942—43. Nine sowings of various types of *Geranium Robertianum* were made. The material was used later for countings of chromosomes (see p. 5). The material collected in nature from a Danish wood (No. 452 Harager Hegn) and two Sealandish stony beaches (Nos. 450—451) was supplemented by

six sowings of seeds from various European botanical gardens; for it was of interest to throw light on the question to how great an extent the clear-cut distinction between var. *genuinum* and var. *rubricaule* noticed in the previous experiments would hold, also when more types within the *genuinum* variety were examined. As several cultures of Danish *genuinum* plants (among them some which have not been mentioned in the preceding two experiments) had not shown any variation worth mentioning within this type, foreign material perhaps might change the picture.

Already the first year the plants appearing could without any difficulty be divided into three widely different types: (1) No. 455 from Lisbon. An extremely small-flowered annual race, which clearly belonged to ssp. *purpureum* and within this, if anything, to the variety *minutiflorum* (cp. KNUTH). It had yellow stamens and the limbs of the petals were a little shorter than their claws. As mentioned above it further had half as many chromosomes as the other types. (2) Nos. 450 and 451 with dense, low, rather small-leaved rosettes; typical *rubricaule* plants. (3) The other six numbers, all with vigorous, large-leaved rosettes; typical *genuinum* plants. The mentioned differences between the three main types appear from Figs. 5—7.

The 20 seedlings of each cultivation number were divided into two groups, one consisting of 10 plants, which were forced as much as possible (in a forcing frame) in order to try whether by this means flowering could be induced already in the first year. In August 1942 the forced plants of no. 455 were already at the fruiting stage (Fig. 6), while the control plants (Fig. 5) were still flowering. All the others were in a stage of invigoration; but two types, viz. no. 450 from Klintebjerg and no. 448 from Cluj (Figs. 5—7) produced a few flowering shoots in a number of the pots. This also holds good of a few of the control plants. Among these some individuals died during the winter, which shows that the principal type of *Geranium Robertianum* can only exceptionally be summer-annual.

In the winter 1942—43 ten forced plants of nos. 451, 452, and 454 were used in an experiment on the dependence of flowering on the winter climate (cold, length of the day, etc.). Four plants of each number were completely withdrawn from the

Fig. 5. No. 455 (ssp. *purpureum* var. *minutiflorum*) and no. 450 (var. *rubricaule*). Flowering plants from the groups not forced. B. phot. Aug. 27, 1942.

winter cold by being moved into a hothouse on the 12th of October, three were not moved in until the fourth of January and three only on the tenth of February. Also three groups of seedlings of the annual no. 455 were included in this experiment. The seeds had been sown in September.

The result of the experiment appears from Table 3. It is seen that it was possible to ascertain a considerable biological difference between *rubricaule* and *genuinum* plants. The former have

Fig. 6. On the left no. 455 from Lisbon at the fruiting stage with withered rosette leaves, on the right no. 448 from Cluj with a few flowering shoots and several withering rosette leaves. Plants from the forced groups. B. phot. Aug. 27, 1942.

a more pronounced winter-rest than the others (cp. SØRENSEN's experiments with *Ranunculus acer*) and cannot be forced to flowering until the end of April, nearly a month before the flowering takes place outdoors. Var. *genuinum* on the other hand starts flowering as early as the beginning or the middle of March. The vegetative development in the form of formation of fresh rosette leaves was more pronounced in the two *genuinum* types than in the *rubricaule* plants. The two *genuinum* types, however, were far from being alike. In no. 452 the group which had been

Fig. 7. From left to right var. *genuinum* (No. 452), var. *rubricaule* (nos. 451 and 450). Nos. 451—52 in rosette, no. 450 with a few flowering shoots. The tape measure extended over 20 cm. B. phot. Aug. 27, 1942.

moved in on the 12th of October began flowering first, while in no. 454 the group moved in on the 1st of January was the first to do so. The plants moved in first were most vigorous in no. 452, but weakest in no. 454, where, again, the group from the 10th of February was most vigorous. During the hibernation a very slow development of a few large rosette leaves took place in no. 452, while in no. 454 many quite short-stalked and small leaves developed, which came to be placed as a small dense rosette within the large one from the autumn. Probably much of the strength of the plants accumulated during the summer was spent in this development of leaves. At any rate the plants moved in first withered and died remarkably soon. It was interesting to compare the annual no. 455 with the others. Already in March its vegetative development was very pronounced, so pronounced that at the end of the month it had large buds. It even managed to be flowering while the *genuinum*

Table 3.

Field No.	Moved in from the open	Date of Observations[1]								Beginning of flowering out doors	Ratio Breadth : Height 19th May	Diameter of flowers cm
		Feb. 2	Mar. 8	Mar. 16	Mar. 28	April 6	April 12	April 27	May 19			
451. var. *rubricaule*	12th Oct.	v	v	v	v	(b)	b(f)	f!	f,fr	21th May	2.3	1.2
	4th Jan.	v	v	v	v	v	b	f!	f,fr			
	10th Feb.	v	v	v	v	v	b	b,f	f!fr			
452. var. *genuinum*	12th Oct.	b	f	f!	f;	f,fr	fr	fr	wi	4th May	0.8	1.1
	4th Jan.	v	(b)	b	f!	f	fr	fr	fr			
	10th Feb.	v	v!	b	f	f!	fr	fr	wi			
454. var. *genuinum*	12th Oct.	(v)	b	b	f!	fr	wi	wi	d	10th May	1.1	1.3
	4th Jan.	v(b)	b	f	f!	f	f,fr	fr	wi			
	10th Feb.	v!	v!	b	f!	f!	f,fr	fr	fr			
455. ssp. *purpureum*	12th Oct.	v	v!	b	b	f!	f!	fr	wi	Control plants all dead	1.0	0.66
	4th Jan.	v	v!	(b)	b	f!	f!	fr	wi			
	10th Feb.	(v)	(v)	(v)	b	f!	f!	fr	wi			

[1] Abbreviations: v = vegetative; (v) weak plants; v! = in vigorous growth. — b = budding. — f = flowering, f! = culmination of flowering. — fr = mainly in a stage of fruiting and with few flowers. — wi = withering. — d = dead.

plants were in full flower, and it was at the fruiting stage when the *rubricaule* plants began flowering. The group from the 10th of February was hurt by the cold, and two of the plants died. All the control plants in the open died, even in spite of the fact that the winter was mild. In the hothouse the temperature during the greater part of the experiment was 6—10° C.

Several features of interest appear from the measures of the control plants (Table 4). In the first place the difference between the beach type and the wood type is more pronounced. Not one of the six sowings of *genuinum* from five different countries approach the measures of var. *rubricaule*. The rather slight variation within the *genuinum* type is remarkable. The mean value for the height, e.g., only fluctuates between 30 and 34 cm, and for the breadth between 62 and 66 cm. Still, we should not be induced to believe that the cultivated types are practically alike. Thus no. 453 was particularly coarse and big, no. 467 had a very open structure of shoots, with vigorous thick stems, while the comparatively small leaves in no. 448 formed a dense hemisphere. This number was particularly downy. Finally we

Table 4.

Field No.		Locality	Length of longest rosette leaf stalks, cm. 1942		Height in cm, 20th May 1943		Breadth in cm, 20th May 1943		Ratio Breadth : Height May 1943	Length × breadth of largest leaves 1943	Diameter of flower in cm, 1943		Life form (Duration)
			Mean	Range	Mean	Range	Mean	Range			Mean	Range	
450	v. *rubricaule*	Klintebjerg ..	8.9	(7—11)	7.3	(4—11)	21	(10—35)	2.9	9.0	1.2	(1.1—1.3)	☉
451		Ll. Vrøj.....	9.8	(7—12)	7.1	(5—9)	20	(18—23)	2.8	7.5	1.2	(1.1—1.3)	☉
448	var. *genuinum*	B.G., Cluj	16.9	(13—20)	33	(30—37)	64	(58—70)	1.9	26	1.3	(1.2—1.5)	☉
447		B.G., Cracow.	16.0	(15—18)	33	(23—46)	65	(47—84)	2.0	35	1.5	(1.4—1.5)	☉
453		B.G., Nancy..	21.0	(17—25)	34	(30—38)	66	(54—80)	1.9	47	1.5	(1.3—1.6)	☉
454		B.G., Ghent ..	15.9	(14—18)	30	(25—36)	65	(50—85)	2.2	37	1.4	(1.1—1.4)	☉
467		B.G., Leyden.	15.4	(12—18)	33	(26—39)	62	(43—68)	1.9	30	1.4	(1.3—1.4)	☉
452		Harager Hegn	17.5	(14—21)	32	(30—34)	62	(50—80)	1.9	37	1.3	(1.1—1.3)	☉
455	ssp. *purpureum*	B.G., Lisbon .	11.0	(8—14)	26	(19—32)[1]	not measured		—[2]	35	0.6	(0.6—0.7)[1]	⊙

[1] Measured in 1942.
[2] The value would be at about 2.
B.G. = Botanical Gardens.

should add the biological differences between nos. 452 and 454 mentioned above.

4. The Cross var. *rubricaule* × var. *genuinum*.

The great difference between var. *rubricaule* and var. *genuinum* invited a closer investigation of their mutual relations by means of a crossing. As starting material no. 450 (var. *rubricaule*) and no. 467 (var. *genuinum*) were chosen, see above. The F_1 generation comprised 15 plants. At the rosette stage the F_1 plants could

Fig. 8. F_1 plant of the cross no. 450×467. B. phot. June 1944.

only with difficulty be distinguished from var. *rubricaule* (the stalks of the rosette leaves reached an average length of 9 cm. (6—12 cm)), whereas the flowering plants were clearly different from both parent races, although var. *rubricaule* characters were most in evidence. The F_1 plants were rather strong as a result of heterosis; in June they were on an average 11.1 (9—16 cm) high and 53.3 (46—65) broad. The ratio breadth : height was on an average 4.9 (4.0—5.9) and breadth × length of the largest leaves of stems was 21 (14—30). The diameter of flowers measured 1.5 (1.3—1.8). The structure of shoots was prostrate with vigorous stems (Fig. 8). All the plant were remarkably similar.

The F_2 generation comprised 302 individuals. The measurements of the plants were made in June, some supplementary measurements, however, being made at the end of August. In June the difference between the individuals was most distinct. Alongside of the measurements some notes on the structure of shoots and on colour were made. Earliness was not ex-

amined thoroughly, as the flowering season may begin at different times in the different years, and unfortunately parent plants, F_1 and F_2, were not cultivated the same year. The three generations moreover were kept under conditions so nearly identical as possible, the plants being cultivated in pots of the same size and being watered in the same way.

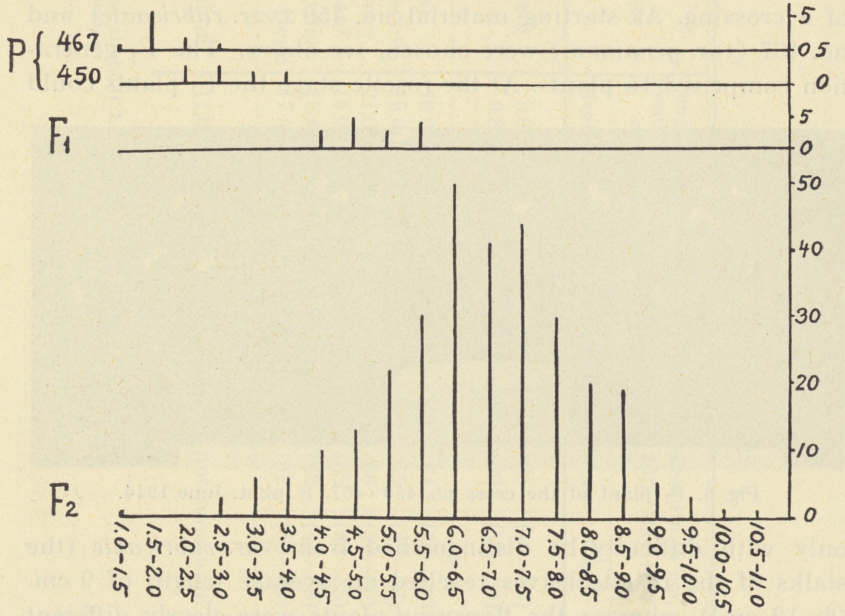

Fig. 9. The breadth:height values in the cross no. 450—467. Abscissa: breadth: height. Ordinate: number of individuals. The measurement of the parent types was made in May nearly three weeks before the measurement of F_1 and F_2, and hence the values for the prostrate no. 450 are too low as compared with F_1 and F_2. According to my notes from August they may at most reach values between 3 and 4.7. For no. 467 the time of measurement has practically no influence.

To begin with the variation in F_2 seemed to be remarkably small, practically all the plants being prostrate. In June, however, a considerable segregation became visible, by which even a couple of characters which had formerly been considered of small importance came to stand out clearly. Fig. 9 shows the ratio breadth : height in the three generations. Here we see an increase of the values from generation to generation. The breadth of the plants increases in relation to their height. This is probably due to dominant genes for prostrate growth being com-

bined with genes for vigorous growth. The former originate from var. *rubricaule*, the latter from var. *genuinum*. The measures of height and breadth were as follows:

	Height			Breadth		
	450	×	467	450	×	467
P	7 (4–11)		33 (26–39)	21 (10–35)		62 (43–68)
F_1	←11 (9–16)			53 (46–65)→		
F_2	← 8 (4–16)			50 (20–77)→		

Fig. 10. F_2 individual (no. 450×467) with thin prostrate branches and leaves both at the base and away from it. This and the following pictures taken in June 1945. The tape measure extended over 20 cm.

The prostrate growth of the majority of plants can be adequately explained by assuming four pairs of dominant genes for prostrate growth. These, however, can only have a completely dominant effect when more than one of the pairs are present at the same time. Among 256 individuals only 12 will have one of the pairs or one dominant gene and a single individual none of the genes being completely recessive. The segregation yielded 288 prostrate and 14 arcuately ascending individuals. There were no individuals having almost erect shoots as in no. 467. The theoretical ratio is very close to these figures, viz. 287 prostrate, 13 arcuately ascending individuals and 1 erect one.

Figs. 10—13 show four F_2 plants. Fig. 13 shows the most characteristic of the arcuately ascending individuals. These may be assumed to have the formulas:

$P_1P_1p_2p_2p_3p_3p_4p_4$, $P_1p_1p_2p_2p_3p_3p_4p_4$,
$p_1p_1P_2P_2p_3p_3p_4p_4$, $p_1p_1P_2p_2p_3p_3p_4p_4$, etc.,

P denoting polymeric genes for prostrate growth. As P_1, P_2, P_3, and P_4 need not be quite alike in their effect, and as these genes may be present both homozygotically and heterozygotically, it is easily understood why the arcuately ascending individuals are not equally characteristic (forming a different angle in relation to the ground).

Fig. 11. F_2 individual with accumulation of leaf-bearing short stems near the base. With thin stems.

Fig. 12 shows a plant whose first internode is erect, but short. After branching follow horizontal internodes, which finally after further branching press against the ground. Of this type there were in all 7(—8) individuals. The character with a first erect internode originates from no. 467. This character is obviously the result of definite, rare combinations of the P genes, but the internode never becomes long as in no. 467. The type p_1p_1 $p_2p_2P_3p_3P_4p_4$ or other types with two different dominant genes will e. g. theoretically appear in 5 out of 302 individuals, a figure which is not far from the 7(—8) found.

As appears from Figs. 10—13 there is a difference with regard to the stiffness and thickness of the stems. Thick, stiff stems as in no. 467, are not common; but intermediary indivi-

Fig. 12. F_2 individual with erect first internodes. Vigorous, stiff stems and no accumulation of leaves at the base.

duals, which resemble the F_1 plants, are of frequent occurrence, and the same holds good of the individuals with thin stems. All individuals with erect first internodes had thick and stiff stems and were without numerous leaf-bearing branches at the base (Fig. 12).

All the 13 arcuately ascending plants were very red and without accumulation of leaf-bearing branches at the base (Fig. I3). The greater tendency towards the development of anthocyanin which was observed in var. *rubricaule* in the experiment mentioned on p. 11, is due to genetic factors. In June 165 greenish, 115 intermediary, and 22 reddish plants were counted. The intermediary plants obviously did not constitute a homogeneous

Fig 13. F_2 individual with arcuately ascending stems and small leaves. The individual shows signs of incipient withering, while in the individual in fig. 11 there is a busy new-formation near the base.

group and might be imagined to represent the two groups out of three in a 9 : 3 : 3 : 1 segregation. With 302 individuals such a segregation would theoretically result in 170 : 57 : 57 : 19, which shows good agreement with that actually found. Later, at the end of August, when the plants in a rainy period had produced fresh, green, leaf-bearing shoots, the differences in colour were vaguer, although the red plants might still be recognized.

The accumulation of leaf-bearing branches at the base results in the plants getting a rosette-like central part. This character, however, is only distinct in June. Later the branches grow out and lie on the prostrate long shoots first developed, the height of the plants thus increasing, particularly because flowering branches of a lower order ascend vertically from the shoots. In August heights between 11 and 31 cm[1] and breadths between 60 and 115 cm were measured. The ratios breadth : height varied between 2.4 and 7.3, thus were somewhat lower than in June. As mentioned on p. 15 no. 467 showed an extraordinarily open structure of shoots, without any kind of accumulation of leaves, whereas var. *rubricaule* was highly provided with leaves at the base. Also as regards this character the genes of var. *rubricaule* proved dominant. In June 210 individuals with accumulation of leaf-bearing branches at the base and 92 without this property were counted. This is fairly close to the ratio 3 : 1 (theoretically 226 : 76). In fact the dominance was not complete, many individuals rich in leaves having no proper accumulation, but being foliose both at the base and along the long shoots. Fig. 11 shows a plant with a pronounced accumulation at the base, while Fig. 10 shows a plant which was equally branched and leaf-bearing at the base and outwards.

The size of the leaves both in F_1 and F_2, if anything, is intermediary in relation to that of the leaves of the parent races. However, individuals with small leaves are in the majority, which indicates that genes for small leaves have a stronger effect than corresponding genes for big leaves.

The diameter of flowers : In F_2 large- and smaller-flowered in-

[1] The highest of these, in all 8 plants, differed from the rest by a tendency towards sending out its secondary shoots obliquely upwards from the former rosette-like centre. Here, too, we may have to do with a character resulting from definite, very rare combinations of the P-genes (cf. what was said above on plants with an erect first internode).

dividuals are seen side by side. The measurements show that flowers with a diameter of 1.3 cm are most frequent, but the segregation ranges from 1.1 to 1.7 cm. Comparisons between the generations as regards this character is not possible, as exterior conditions have a great influence on the diameter of the flowers. Thus the flowers coming out first in the spring, and flowers in periods of damp weather are the largest. For the same reason a comparison of the earliness of the generations had to be disregarded. There was a clear difference between the F_2 plants, some of them flowering as much as a week before the others.

The crossing experiment shows that the two varieties are distinct by a particularly large number of genes, and that important differences, e. g. prostrate or erect growth, depend on the interplay of four pairs of genes working nearly alike. This obviously agrees with the experience made bay CLAUSEN, KECK, HIESEY, and MARTIN (1943) and CLAUSEN, KECK, and HIESEY (1944), according to which climatic races or ecotypes differ by many characters, and the segregations indicate that even small morphological differences are determined not by one, but by several pairs of genes. Differences in habit, mode of branching, earliness and other characters are frequently governed by three or four pairs of genes.

5. Distribution and Ecology.

The distribution and ecology of var. *rubricaule* offer several points of interest. The total range seems to include seashores from Madeira to Norway[1] and Wormsö in the Baltic. However, it appears from the above experiments that the range based on herbarium studies is by no means well established. To a certain degree the same holds good of the distribution in Denmark. Here, the writer himself, however, has often seen the plants in nature, and is therefore able to state that in Denmark the plant is common only along the coasts of the southern part of the Kattegat (Bay of Sejerø) and the Great Belt.[2] The range in Denmark reminds of the ranges of a number of southern continental plants, and in many cases the habitats of the variety are actually just

[1] OSTENFELD's plants from the Isle of Møgster near Bergen in Norway are very questionable.

[2] In the locality Ulvshale in the Isle of Møn, *G. Robertianum* is abundant on shingle (old raised beaches) in clearings in a wood. All plants have the growth habits of var. *genuinum*. As a result of the habitat, however, they are reddish. Other reddish modifications of var. *genuinum* were observed in mountain screes in Southern Norway and on the stone sett along the western side of the fiord of Præstø (Sealand).

below slopes covered with luxuriant continental grassland, or, as e. g. in the Isle of Reersø, close to the old raised stony beaches covered with a *Seseli libanotis* vegetation. The main type from woods is common in Eastern and Northern Jutland and on the Danish islands (see KØIE 1939). The absence of var. *rubricaule* on many shingle beaches in Jutland may be due to climatic factors. In England, too, the *rubricaule* type has a southern distribution (EVANS).

In the localities examined by the writer the *rubricaule* plants were growing on shingle most frequently resting upon clay or sand rich in organic matter and sometimes giving a positive diphenylamin reaction. The p_H-values found were 7.7 and 8.4 (Klintebjerg) and 7.5 (Fyens Hoved). The soil near the root tips was always rather moist, and thus soil conditions somewhat remind of those of var. *genuinum*, which grows in moist soils rich in organic matter and mostly also rich in nitrates.

Var. *rubricaule* inhabits soils not particularly saline, but grows in the upper part of the beach, where the vegetation consists of non-halophytic species and a few halophytes.[1]

Near Klintebjerg the vegetation includes *G. Robertianum rubricaule!* [! = dominant], *Galium aparine!*, *Lathyrus maritimus*, *Agropyrum repens*, *Crambe maritimum*, *Hieracium umbellatum* var., *Leontodon hispidus*, and *Sonchus arvensis* var. On the Isle of Nekselø, the *rubricaule* variety grows in a community with *Beta maritima!*, *Rumex crispus!*, *Galium aparine!*, *Convolvolus arvensis!*, *Hockenya*, *Crambe*, *Linaria vulgaris*, *Cynoglossum officinale*, *Verbascum thapsiforme*, *Sedum acre*, *Potentilla anserina*, *Daucus carota*, and *Circium lanceolatum*. These vegetations represent transitions from typical maritime beach vegetations to the dry grasslands which are common near the sea on level alluvial soil.

From the north coast of Møn in the Baltic there is a description of a *Geranium Robertianum rubricaule* vegetation which coloured large parts of the stony beach red (BÖCHER 1946 p. 3 and Fig. 2). In this place the vegetation grows close to the woods on Møns Klint [the chalk cliffs of the Isle of Møn], where var. *genuinum* occurs. So there is here a possibility of hybridization between the two varieties and this also may have taken place; for in the wood near the ravine of the Taler there is a colony of rather prostrate individuals, which reminds somewhat of the F_1 plants of the mentioned cross no. 450 × 467. To make sure that these were hereditarily prostrate individuals occurring in a wood, seeds were collected for cultivation in Copenhagen. The new plants (no. 620) were very similar to those from the wood of the Taler. They were 16.5 (10—23) cm in height, 71.5 (40—117) cm in breadth, which gave ratios breadth : height from 2.3 to 6.2 (mean value 4.3). The average figure for the size of the leaves was 25.1 (11—34) and the

[1] TANSLEY (1939, p. 890 and Plate 160) describes the occurrence of *G. purpureum* in England as follows: »A very characteristic plant of stable shingle which is common on many south coast fringing beaches«. I am inclined to consider his *G. purpureum* and var. *rubricaule* as identical (cf. EVANS).

diameter of the flowers 1.2 (1.1—1.3). The plants were markedly coarser and the leaves larger than those of F_2 in the cross produced in the experiment, and further the branches in their extreme parts were ascending more than those of the former. No. 620 gradually grew reddish by cultivation on soil open to light. It had no accumulation of leaf-bearing shoots at the base.

On the south coast of Nakkeland near Rørvig and on the Isle of Bornholm almost erect reddish individuals were observed on a stony beach. Thus prostrate types may appear in woods and erect types on beaches, but deviations from the normal distribution of the races in the two environments are very rarely found.

6. Discussion.

In a paper on *Melandrium album* and *rubrum* D. Löve (1944) advances the view that these species, which are not separated by a barrier of sterility, if anything, are ecotypes. However, she continues, such ecotypes, which are morphologically distinguishable and distributed in different environments, ought to be termed subspecies. Amongst others she supports this view on papers by Clausen, Keck, and Hiesey. Transferred to *Geranium Robertianum* these principles will lead to the dibasic ssp. *purpureum* being promoted to the rank of a species, and var. *genuinum* and var. *rubricaule* being promoted subspecies.

Such a step perhaps will be rash. In the first place it is of practical value to leave such clearly related plants as *G. Robertianum* s. str. and *G. Robertianum* ssp. *purpureum* as subtypes of the same species. The fact that because of cytological conditions they must be supposed to be separated by a barrier of sterility is of less importance; for we know examples of plants which can hardly be distinguished morphologically having different chromosome numbers (17 and 34 in *Campanula rotundifolia* var. *uniflora*, 18 and 36 in *Veronica officinalis genuina*). Further conditions in *G. Robertianum* are complicated by the fact that a dibasic *genuinum* type has also been found here, and hence it is doubtful whether the latter can be distinguished morphologically from the tetrabasic races. If the ssp. *purpureum* and *G. Robertianum* s. str. are still in time to be maintained as independent species, it will probably occasionally be necessary to include them in a collective species, and so nothing is gained. At present, when investigations of the conception of species and

the small units are in rapid development, it does not seem justifiable to make alterations of names or of the systematic classification. It will probably be the best thing to keep experimental and orthodox taxonomy distinct for some time, until problems have been settled through discussions and by the procurement of more material.

As compared with conditions in most other species I have studied, the discontinuity in the variation in *G. Robertianum* in Denmark is of particular interest. Whereas the races in *Plantago maritima, P. lanceolata,* or *Veronica officinalis* form variation series or clines (cf. e. g. HUXLEY 1939, 1942, GREGOR 1939, 1946, BÖCHER 1943, 1944), there are two clearly distinct types, each with a fairly moderate variation, in *G. Robertianum.* This is perhaps due to the fact that there are only two environments, wood and stony beach, suitable for this species. It is, however, remarkable that a species which may form groups of races adapted to so widely different kinds of localities, has not developed other ecological varieties, e. g. races connected with cultivated soil exposed to light or with accumulations of seaweeds on the beach.

Only in *Hypericum pulchrum* (see BÖCHER 1940) I have seen a similar case of discontinuity in the variation. Here the North Atlantic var. *procumbens* Rostrup is clearly distinct from the erect principal type. The clear-cut distinction perhaps may be explained as due to isolations of the *procumbens* type during the Glacial Age. I remind of this fact here because it is not at all excluded that the distinction between the *Geranium* varieties has arisen very long ago. At an early stage there may have been a continuity in the variation, after which a selection of the wood and beach types took place, and because of the great difference between the two environments these types have kept fairly distinct and have each been able to maintain its line of development. A more thorough discussion of this problem, however, must be postponed until the *Geranium Robertianum* populations of other regions have been examined in detail. Particularly the populations on the British Isles and in Southern Europe seem to include types which migth be of interest in an attempt at ascertaining whether there is nowadays a discontinuity in the variation everywhere.

7. Summary.

(1) In *Geranium Robertianum* s.l. the chromosome numbers 2 n = 32 (ssp. *purpureum* var. *minutiflorum*) and 64 (6 different samples of var. *genuinum* and 2 of var. *rubricaule*) were found.

(2) Relations between var. *genuinum* and var. *rubricaule* have been investigated through three cultivation experiments under varied exterior conditions. The two varieties in all experiments proved to be widely different. They may be described as follows:

var. *genuinum*: erect, large-leaved, with rather large flowers and early flowering and less pronouned winter-rest. Connected with woods, particularly on humid soil rich in humus and nutritive substances.

var. *rubricaule*: prostrate, small-leaved with somewhat smaller flowers, late-flowering, and with pronounced winter-rest. Connected with the upper part of the stony beach, particularly in warm localities and where under the pebbles there is clay or humus with sand added.

In both varieties the length of the root and the red colour of the plants are highly due to exterior conditions.

(3) The varieties, when crossed, produce a hybrid, which resembles var. *rubricaule* most. In F_2 the majority of the individuals are prostrate. The difference between the varieties is mainly due to four pairs of genes producing prostrate growth.

(4) Both varieties have a biennial development when the seeds are sown in spring. Still, some individuals in a few races can be forced to flower in the first summer (summer-annual development). In nature the seeds germinate from the spring until late in summer; the plants germinating last become winter-annuals.

(5) The South-European dibasic type when cultivated outdoors was summer-annual. Plants sown in the autumn died in winter, but developed winter-annually in the hothouse at 5—10° C.

(6) The variability within each of the two types var. *rubricaule* and var. *genuinum* is fairly slight. The great ecological and morphological barrier between them is interesting in comparison with species having a continuity in variation.

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